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Pollen and Pollination

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Preface

Pollen studies make important contributions to our knowledge in many interdisciplinary arenas. Pollen identification is widely used in reconstruction of, e.g., vegetation, the climate of the past, and plant biodiversity. Studies concerning pollen structure, size and form are key issues in basic sciences, as, e.g., plant taxonomy and evolution, but are also of importance in applied fields as, e.g., plant breeding. In pollination studies pollen is generally used specifically to identify food sources of visitors and to reconstruct their foraging routes. Fewer have been devoted to pollen collection mechanisms and to the structure and content of pollen in relation to its function.

A computerized search of the current literature reflects these general trends. A search done on December 1st 1999 showed the following: In *Agricola* the term pollen was mentioned 13354 times, pollination 7184, the combination pollen + pollination 36, and pollen + ecology 17. In *Biosis* the results were: 15515, 3985, 57 and 3; and in *Bioscience* 53886, 20187, 502 and 38, respectively.

The present volume, filling a gap, is an attempt to produce a comprehensive interdisciplinary text as a bridge between scientists who study various aspects of pollen biology: physiologists, botanists, ecologists, zoologists and neurophysiologists. We aim to consider the functional ecology of pollen in relation to its structure and constituents as a framework towards a better understanding of evolutionary processes that mold pollination biology.

The 16 papers, all of which were commissioned especially for the present volume, could be roughly classified, by their interdisciplinary

nature, into three main themes: pollen structure and constituents, pollen evolutionary ecology and the pollen-pollinator interface. Several papers overlap somewhat or are perhaps even somewhat contradictory and reflect the author's own ideas and experience. Some could be understood more deeply by consulting other closely related articles. The reader is strongly referred to the respective literature list of each article.

The last steps of anther ripening and pollen development (Pacini) and the mature pollen wall structure (Hesse) are key factors to understand pollen dispersal mechanisms in biotic pollination (Stroo) as well as abiotic pollination (Ackerman). Pollen size, shape, wall stratification and the micro-topography of its surface as well as the composition and structure of the pollenkitt have an important role in pollen-connecting mechanisms (Thorp) and also in the dispersal mechanism in which electrostatics is an important factor. Pollen electrostatics (Vaknin et al.) is reviewed from theoretical as well as practical aspects in relation to natural and agricultural systems. Special attention is devoted to the importance of the flower's morphological features that confer adaptiveness to electrostatic pollination.

Abiotic pollination is discussed by Ackerman, who surveyed definitions and terminology of the phenomenon. Pollen physical characteristics are evaluated in relation to its functional ecology and the evolutionary relationships between plant morphology and fluid dynamics. His findings are closely related to those on pollen structure (Hesse), electrostatics (Vaknin et al.), and content (Pacini, Nepi and Franchi) as well as to viability and longevity

(Dafni and Firmage) and adaptation to special pollinators (Stroo).

Pollen constituents are viewed from two complementary angles: pollen cytochemistry (Nepi and Franchi) and pollen nutritional value (Roulston and Cane). Pollen cytochemistry (Nepi and Franchi) deals with the application of cytochemical methods to mature pollen with special reference to reserve material. The chapter on pollen cytochemistry serves as a basic background to understanding pollen physiological behaviour and chances for germination and fertilization. The chemistry of the pollenkitt is a key factor in understanding pollen dispersal (Ackerman; Hesse et al.) as well as in pollen odour (Dobson and Bergström) and colour (Lunau). The inner pollen composition is related to its viability (Pacini; Dafni and Firmage) as well as to its value as a food for consumers (Roulston and Cane).

Pollen viability and longevity is discussed from practical, ecological and evolutionary viewpoints. The article by Dafni and Firmage surveys the advantages and disadvantages of the existing methods to asses pollen viability and examines the current hypotheses that link pollen longevity and pollination biology.

Pollen may attract flower visitors by visual (Lunau) as well as by odoriferous cues (Dobson and Bergström). In general, our attention is focused on petals (and sepals) as the main attractants of flowers. Lunau draws our attention to the importance of visual pollen signals. While considering evolutionary, ecological, sensory-physiological and behavioral aspects of flower-pollinator interaction, the various strategies of angiosperms for attracting pollinators to the site of rewards are elucidated with special reference to pollen. Floral odours are important cues even as a long distance signals that flowers emit. Dobson and Bergström discuss pollen odours in relation to bee foraging behavior, location of pollen sources, discrimination of pollen amounts and specific recognition of flower resources. Their work includes a review of the current knowledge as well as new experimental

evidence and chemical analyses concerning pollen odours.

Cruden deals with the intriguing question of why there are so many pollen grains? This article examines the selective forces that affect pollen number. The author analyses the relationships among other floral traits of animal-pollinated plants, including pollen size, stigma area and depth, and the pollen-bearing area of the pollinator that may affect pollen number, and also provides a model to examine how change in one trait may elicit change in other traits.

Pollen as a food for its consumers is a widely neglected topic. The article by Roulston and Cane covers the mechanisms of pollen digestion by animals; the efficiency of removal of pollen content and the taxonomic distribution of pollen content. All these aspects are discussed in relation to adaptive hypotheses that associate pollen chemistry with pollinator rewards. Roulston and Cane surveyed the digestion and nutrient content of pollen, which is an outcome of pollen cytochemistry (Nepi and Franchi). They discuss mechanisms of pollen digestion and its efficiency and the range and taxonomic distribution of pollen nutrients. All these address adaptive hypotheses concerning pollen chemistry and pollinator reward. Pollen digestibility is, to a large extent, an outcome of the pollen wall structure (Hesse) and content (Pacini).

The various mechanisms of pollen collection by bees are surveyed by Thorp. Pollen is an essential food of many pollinators especially bees due to its nutritive contents (Roulston and Cane; Pacini) especially bees. Thorp surveys the various structures and behavioral adaptations for acquiring and transporting pollen by bees.

Pollen structure and packaging have co-evolved in relation to transfer functionality and pollinator's specificity. Stroo reviews the pollen morphological evolution in bat-pollinated flowers. An analysis of pollen size, shape, aperture number and type and ornamentation type is used to test previous hy-

potheses concerning the characteristics of bat-pollinated pollen. Orchid pollinaria structure is discussed in relation to its functionality (Johnson and Edwards). Aspects as attachment to vectors, mechanisms of pollen deposition, pollen longevity (see also Dafni and Firmage), pollen:ovule ratio (see also Cruden), spatial dispersal patterns and outcrossing in relation to pollen export are also discussed. This contribution is a key to elucidating Roubik's paper on a new deceptive mechanism in orchids involving Meliponini bees as pollinators. It is assumed that the relationship is based either on mimicry of rewarding resources or on a bee pheromone.

Various thread-forming structures in pollen anthers are discussed by Hesse et al. in relation to their role in pollination ecology. These unique structures may function as pollen-connecting vectors in forming pollen dispersal units and/or playing a role in pollen presentation.

Beetles are important pollen-eaters, Bernhardt reviews the convergent evolution and adaptive radiation of beetle-pollinated angiosperms. His survey stresses the diversity of beetle-pollinated flowers and their shared and peculiar adaptations contributing to the syndrome of cantharophily and its evolution.

We would like to acknowledge here all those who have made possible the publication of this volume. We are very grateful to all our contributors for their effective co-operation, and to all our reviewers for their careful revisions and for their efforts to return quickly their comments and suggestions. We would like to thank especially the staff of our publisher, Springer-Verlag in Vienna, for the help and support during the preparation of this special issue of *Plant Systematics and Evolution*. It is hoped that this interdisciplinary volume will promote multifaceted studies leading to a better understanding of pollination evolutionary ecology. Floral rewards, such as pollen and nectar, are only one aspect of the multidimensional pollination kaleidoscope. Any attempt to evaluate the role of pollinators attraction and behaviour has to be considered in relation to the reward's content, composition, and physical and chemical properties, all this in relation to environmental conditions as well as the agent's efficiency as a disperser of a functional pollen.

A. Dafni, M. Hesse and E. Pacini,
Guest Editors

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Pollen wall stratification and pollination

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Abstract. Qualities of the stratified pollen walls were evaluated for their possible role in pollination (pollination modes, and pollen tube formation). The importance of studying pollen grains in their respective natural state is noted. Examples of pollen morphological features specific to pollination vectors are rare and difficult to demonstrate. However, some complex, but significant correlations are reported.

Key words: Angiosperms, pollen, pollen wall, pollination, exine, intine, aperture, ornamentation.

General comments on the topic

The question of whether and to what extent the features of the (angiosperm) pollen wall reflect adaptations to the respective pollination mode is not a new one, but it remains unsettled. In a previous conference on pollen form and function (Blackmore and Ferguson 1986) mostly problems of correlations between the easily accessible surface of pollen grains and the respective pollination mode were addressed: e.g. the contribution by Thanikaimoni (1986) in this volume on apertural adaptations to the modes of pollination. More facts and ideas were presented in a later pollen conference in 1990 (Blackmore and Barnes 1991). At that time, details of pollen wall stratification and its possible relation to polli-

nation were barely debated. Meanwhile, new data provided more information on relations between sporoderm features and pollination. To my knowledge, the present review is the first to address the features of the sporoderm (i.e. qualities of the stratified pollen wall) and their possible relation to pollination, whereas several previous original papers and reviews have addressed the possible correlation of surface features with the various pollination modes. Concerning approaches to polyad function, for reproductive effort and success and for polyad function expressed through pollen wall morphogenesis, the reader is referred to the classical review by Knox and McConchie (1986).

It should be stated that we are far from a complete understanding of all correlations of pollen surface features with pollination vectors. Similarly, the role of the various sporoderm strata in this context is poorly understood. Some of the major reasons deserve a special note:

1. Muller (1979, p. 628) hits the point: "The large variability of angiosperm exine morphology probably reflects compromises between the different functional aspects, often with one function dominating", and additionally (p. 595): "Basically, any exine structure is a compromise between the protective, the harmomegathic, and the reservoir function".

2. In many taxa (even at the family level), too little is presently known about pollination vectors (such as in many Araceae genera without any trap-like features, e.g. *Pothos*, *Gymnostachys*). Significant correlations between pollen vectors and the manifold, probably relevant pollen-/anther structures are rare. Some of the numerous sceptic statements are collected here. Ferguson and Harley (1993, p. 239 ff): "Based on comparisons of pollen morphology, floral and plant morphology, ecology and pollination vectors within the Palmae, it would appear that pollination within many species of palms is effected by the interaction of a number of floral strategies such as phenology, temperature, odour, nectaries and colour. It seems likely that examples of pollination vectors specific to pollen morphological types will prove to be rare and difficult to demonstrate". This statement may be valid not only for the palms, but could also be extended to nearly all angiosperm groups. In this context the interesting remark by Osborn and Philbrick (1994, p. 263) is relevant: "Although the functional association of pollen morphology and ultrastructure to particular pollination syndromes is not always related (Taylor and Levin 1975) nor well-understood, a number of angiosperm families have in fact been shown to demonstrate positive correlations between these palynological characters and their pollination systems...".

3. Another important point to be stressed: the (mature) pollen grains should be studied preferably in their respective natural state. Many attempts were made in the past to correlate features of artificially cleaned (acetolysed) pollen with functional aspects. After acetolysis, all non-resistant components are gone, many of them of high relevance for correlations (Hesse and Waha 1989). In pollen with a thick, massive exine (e.g. Asteraceae), this factor does not play a crucial role, but more taxa than supposed in mind have more or less delicate sporoderms (Furness and Rudall 1999). Rowley et al. (1997) pointed out that the skin-like exine of mature Strelitziaceae pollen is similar to the sporopollenin coating

on some algae: both skin-like coatings probably do have functions in spite of their extreme thinness; the coatings may prevent infection and resist enzymes, but facilitate pollen tube formation by its omniaperturate condition.

4. It is clear that we should be careful in assigning any function to spectacular features, as is illustrated by two examples: The first one related to the unsolved problem of a possible function of the orbicules which constitute a spectacular feature. Huysmans et al. (1998) summarized the theories on possible function of orbicules. The second one concerns the apertural protrusions in *Geranium* (Geraniaceae), which, at least up to now, do not have any recognizable function (Weber 1996a). The situation for the so-called pollen buds in some Rubiaceae is somewhat different: Weber and Iggersheim (1994) found a correlation between the detachment of the protrusions from the pollen grains and the release of pollenkitt from the tapetum. However, the "pollen buds" in many Rubiaceae were misinterpreted as "protruding onci" by Tilney and Van Wyk (1997).

Correlations

Although examples of pollen morphological features specific to pollination vectors are rare and difficult to demonstrate, the following examples include some complex, but interesting correlations.

A. Relations between ornamentation/sculpture and pollination

Keith Ferguson (together with co-workers) has published examples of correlations between pollen morphology and pollination mode. An excellent literature review concerning the association between pollen *morphology* and pollination mechanisms is found in Ferguson and Skvarla (1982). Most of the examples, however, concern only the correlations between pollen *ornamentation* (i.e. the pollen surface) and pollination mechanisms. Ferguson and Skvarla (1982, p. 190, 191) mention only a few examples of a relationship between

internal structures and the actual pollination mode: e.g. *Alexa* and *Castanospermum*. Ferguson and Skvarla (1982, p. 191) state, however, that the role of tectal columellae is well-known, whereas the evolutionary significance is not clear. The tectal columellae might be of some phylogenetic importance. The function of these structures is undoubtedly complex, but one function is certainly the accommodation of pollen surface loadings. (For details see below under chapter B.)

Attention should be drawn to the study by Hemsley and Ferguson (1985): *Erythrina* (Leguminosae) pollen is more crude in passerine-pollinated taxa and exhibits more pollenkitt than the pollen of the hummingbird-pollinated taxa. Finally, Ferguson and Pearce (1986, p. 294) indicate a clear correlation between exine ornamentation, floral morphology and pollination in *Bauhinia*. Also in other families, such as Cabombaceae, where all palynological characters correlate well with the anemophilous syndrome of *Brasenia schreberi* and the entomophilous *Cabomba carolineana*, respectively (Osborn et al. 1991). However, other questions are far from being settled. The remarkable pollen structures of *Pinanga* (Palmae) raise the question of functional significance, and it is speculated that beetles are the pollinators, but very limited data are available (Ferguson et al. 1983).

The complex question of functional significance is highlighted by features found for example in Araceae or conifers. Grayum (1986, p. 324) clearly states that exine sculpturing is not the only pollen character that may be affected by pollinator selection. It has been documented that the larger types of beetles probably select for larger pollen in some Araceae. Further examples were published by Grayum (1992): a) psilate/verrucate ornamentation (e.g. in *Philodendron*) correlates with beetle pollination, b) spinose ornamentation – many Aroideae – strongly correlates with pollination by flies, c) columellate/reticulate ornamentation (e.g. in Monsteroideae) weakly correlates with pollination by bees; an interesting idea from van der Ham et al. (1998, p. 135)

suggesting that polypligate pollen in Araceae – e.g. in *Amorphophallus* – may be correlated with the chemistry of female parts of the spadix, and pollination by small beetles, e.g. Nitulidae. The sacci of most conifer pollen grains, often interpreted as structures that aid only in wind dispersal (Niklas 1984) or pollen orientation on the nucellus, function primarily as floatation (and gravity) devices (Tomlinson 1994). In *Picea orientalis*, the sacci are porous, and pollen does not remain buoyant in contrast to *Picea glauca*, where air pockets in the saccal ektextine remain buoyant; structural modifications of this sort may influence speciation (Runions et al. 1999). A curious report comes from Linder and Midgley (1996): anemophilous plants (*Pinus*) may select pollen from their own species from the air. In this context the reader is referred also to Owens et al. (1981) for relationships between pollen structure and pollination mechanism.

Another strange characteristic is the cohesion strands, a term coined by Burns-Balogh and Funk (1986). Referring to neottoid and spiranthoid orchids, as for example in *Schiedeella*, the cementing, acetolysis-resistant, sporopollenin substance of the tetrads may assume a thread-like appearance when the tetrad members are teased apart. According to Dressler (1993), this curious substance may act to reinforce soft pollinia. The cohesion strands should not be confused with the genuine viscin threads found in Onagraceae or Ericaceae. In this context a few remarks about (sporopolleninous) viscin threads would be appropriate: Skvarla et al. (1978) state that beaded viscin threads are associated with bird- and moth-pollinated taxa, and smooth and simple threads occur in bee-pollinated taxa of Onagraceae. Further details of form and function of structures assuming a thread-like habit (not only viscin threads) are found in Hesse et al. (this Plant Syst. Evol. Volume).

B. Relations between pollen wall stratification and pollination

The reports are listed according to the various sporoderm wall strata, starting with the out-

ermost layer. The Figs. 1 and 2 show the extremes in sporoderm configuration: a cavate, tectate, columellate *Argyranthemum* pollen (with an infratextum) and an intectate *Pistia* pollen. Examples that do not correlate with a distinct stratum are presented at the end of the paper.

Ektexine

The *Tectum* (or the sculptured ektexine surface in atectate pollen) is involved in pollination as shown above.

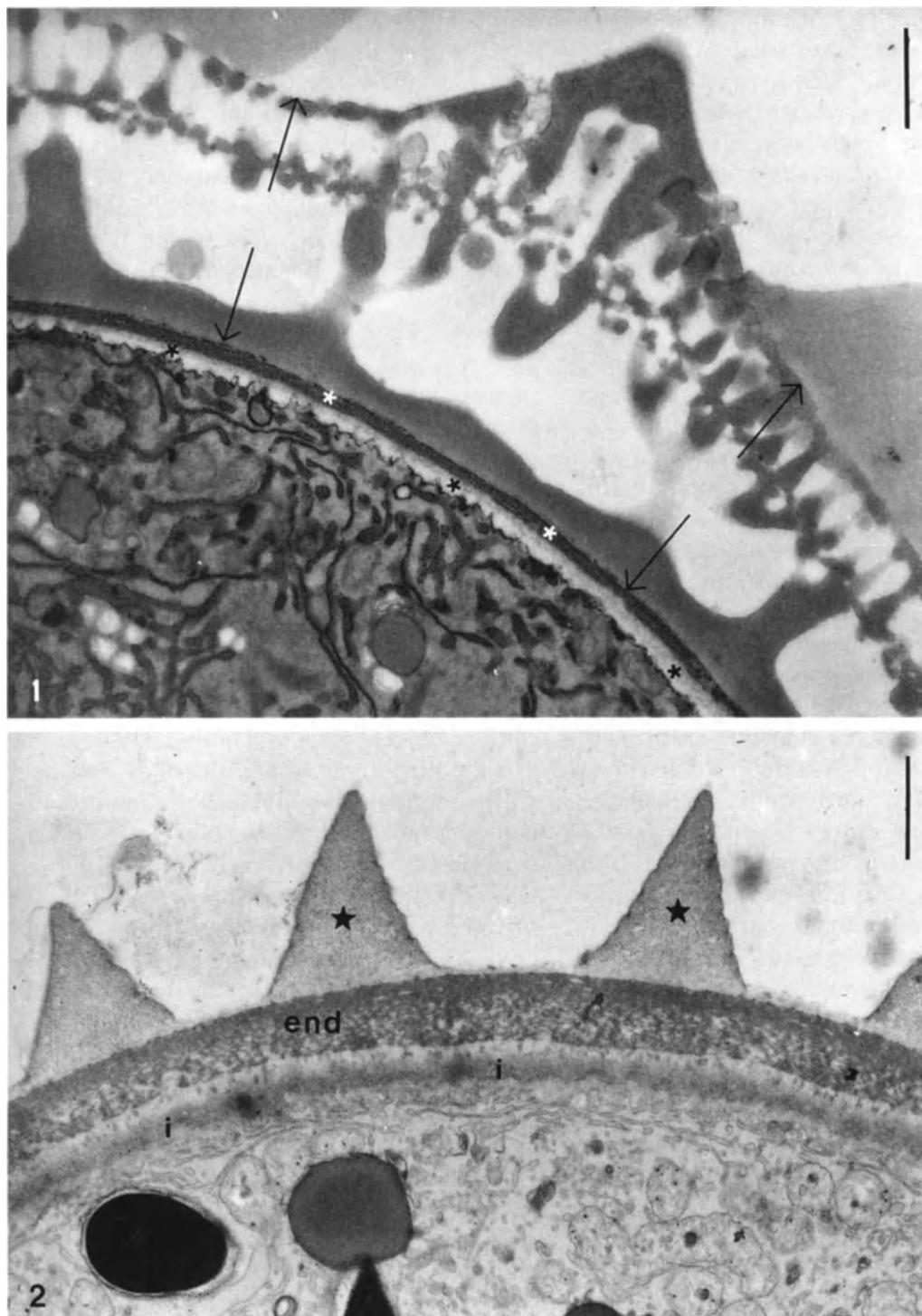
One should not assume that it is incorrect that anemophilous taxa have only a simple stratification as compared with zoophilous pollen, see some fern spores with their uppermost, often elaborated stratum, variously called perine, perispor etc. Similarly, assuming that wind-pollinated taxa have exclusively smooth pollen grains comes close to oversimplification (see the case of Lactoridaceae below). Moreover, text books generally consider entomophily and anemophily as strictly different pollination syndromes, which is doubtlessly also an oversimplification. It was, however, recently shown again, that anemophily might be quite common in entomophilous taxa (Mahy et al. 1998, also for review), and probably vice versa. Interestingly, there is indirect evidence for anemophily in the tropical rainforest angiosperm *Myrsine* (Myrsinaceae) with its elaborated, 4-(5-) colporate pollen: this supports the notion that anemophily is not necessarily rare in tropical rainforests (Otegui and Coccucci 1999, also for review).

Electrostatic forces are perhaps directly connected with the outermost layer of the exine. In the past, there was little more than pure speculation (Corbet et al. 1982, Erickson and Buchmann 1983). Only Chaloner (1986) suggests that an unsculptured pollen grain would lose its charge more rapidly to achieve the same charge of the stigma. Galati and Rosenfeld (1998) assume that because exine and orbicules consist of sporopollenin, their surfaces are electrically charged in the same way. This would lead to mutual repulsion and enhance pollen expulsion.

A better studied topic concerns all the “pollen loadings” upon or “in” the sporoderm.

Such “surface loadings” are represented not only by pollenkitt, but also by proteins (see below). There is no evidence to suggest that the pollenkitt material would play any part in recognition reactions. Some explanations of terminology related to *pollenkitt* and other tapetal products may seem appropriate. Thus, pollenkitt means the deposited lipid fraction of the tapetal organelles plastids and ER only, and *tryphine* is the mixed fraction of degenerated tapetal organelles with lipid and cytoplasmic remnants. The more general term *pollen loadings* includes other components besides lipidic fractions and/or cytoplasmic remnants, such as the sporophytic proteins.

The role of pollenkitt has been subject to ongoing debates: Pacini (1997) assigns several primary and secondary functions, including (Pacini, pers. comm.) the following: because some pollen grains have pollenkitt and others do not, bees use the former grains to keep the other grains devoid of this glue. The abundant presence of pollenkitt is mostly a sign for entomophily (also the more rare ant-pollination requires very sticky, rather small pollen, and is related to dry habitats, Wyatt 1981), but in some cases (e.g. Ericaceae: *Calluna*, *Erica*; Primulaceae: *Cyclamen*) the pollenkitt gets dry after some hours, and whitish, and the pollination mode may shift towards anemophily; this is also the case in *Tilia*, and other amphiphilous taxa. This may be in some taxa (not in *Cyclamen*, there is no nectar) related to the end of nectar production (Franchi and Pacini 1996) and to the presence of pending flowers, when the release of dry pollen is facilitated. At the end of anthesis, pollenkitt changes its chemophysical properties, such as color and viscosity, probably to avoid further visits by insects (Pacini pers. comm.). This was observed in *Erica* (Hesse 1979), which, like *Calluna*, is visited first by insects, mainly bees. When nectaries cease to produce nectar, pollen becomes more powdery, and is released easily by the anthers (Pacini and Franchi 1996). The



Figs. 1, 2. TEMs of cross sectioned mature pollen grains. **1.** *Argyranthemum frutescens* (Asteraceae). The ektexine, with tectum, infratectal columellae, infratectum, columellae, and foot-layer, is indicated by the arrows. White asterisks endexine, black asterisks intine. Bar: 1 µm. **2.** *Pistia stratiotes* (Araceae). Exine with acetolysis-susceptible ornamentation (asterisks, in reality ribs, but spiny in cross sections), *End* endexine, *i* intine. Bar: 1 µm

amount and interesting role of pollenkitt in an anemophilous plant, *Mercurialis annua*, was studied by Lisci et al. (1996).

The statement by Márquez et al. (1997a, 1997b) concerning observation in Poaceae of a small amount of translucent, foamy lipid material produced by tapetum may be correct. Pollenkitt is more widespread in angiosperms than generally assumed because it is not easily recognized, in contrast to Gymnosperms totally lacking pollenkitt. Therefore, its occurrence in Poaceae is not so remarkable (Hesse 1980). The accumulation site of pollenkitt may vary and cause problems of detection. It may remain deposited inside the former tapetal cells (*Populus*) or drift to the pollen grains. Pacini et al. (1992) found comparable plastid differentiations in species with or without pollenkitt. However, we call attention in stating "absence of pollenkitt": pollenkitt may be produced also by the endoplasmic reticulum, at least nearby (in *Rosmarinus* it is the only organelle producing pollenkitt: Uberta-Jimenez et al. 1996). Moreover, pollenkitt may tapetally be formed, but will not reach the pollen grains: an intact cytoplasmic tapetal membrane encovers the astonishingly large amount of lipids while the mature grains remain dry (this is the case in *Populus*, Hesse 1979). Pollenkitt is commonly identified with viscous fluids, but not with non-fluid substances. Examples for the latter are carotinoid crystals (Freytag 1958) and lamellate waxy crystals (Dunbar 1967). *Freycinetia* (Pandanaceae) produces waxy corpses on the psilate/punctate pollen surface; the corpses function as sticky material adhering to the hairs of the flying foxes (birds) (Cox 1984).

Pollen coating vesicles, acting as a protective barrier that is later superimposed by pollenkitt, constitute a special example (cf. Weber 1996b), which may - to some extent - be comparable with the location of the sporophytic proteins in pollen coatings, as found especially in the tryphine material of Brassicaceae. [N.B.: Most of work was done in Brassicaceae. However, other taxa besides Brassicaceae and other coating substances deserve investigation. El-Ghazaly and Graft-

ström (1995) presented a good overview of presence and distribution of pollen wall loadings in *Betula*, i.e. outside of Brassicaceae, during dormancy up to anthesis. Polyamines, carotenoids, and other possibly sporoderm-located substances may also play a role in pollination, cf. Bagni et al. (1999), and Giuliano (1999). Data are very scarce at present, much more research is necessary.]

Extensive reviews along with some original results were published by Piffanelli et al. (1998) and Wu et al. (1997). From the biochemical point of view there is a lot of information (e.g. biosynthetic pathways of lipidic structures during development), but the "pure" palynological and cytological aspects are sometimes not up-to-date, as is apparent in the questionable drawings in Piffanelli et al. (1998, e.g. their Fig. 1). Similarly, the fibrils reported by this team are in fact remnants of the primexine matrix, and probably do not come from the tapetum. It is further incorrect to call the "tapetosomes" "novel" organelles (Wu et al. 1997), not referring to Dunbar (1973), who first used this term with this connotation.

A good report of biosynthesis and processing of proteins, forming main pollen coat components, derives from Murphy and Ross (1998): "... many of the pollen coat proteins [in *Brassica* tryphine] derive from an endoproteolytic cleavage of precursor oleosin-like proteins that originally accumulate within the large cytoplasmic lipid bodies of tapetal cells The cleaved oleosin-like proteins may also play a role in stabilizing the various pollen coat components on the pollen wall and in facilitating pollen rehydration and germination."

This is further substantiated by Stephenson et al. (1997), indicating that the male determinant of self-incompatibility in *Brassica* [tryphine!] is located in the pollen coating. "Upon pollination, the coating flows out from the pollen exine onto the stigma surface, forming a characteristic 'foot' which penetrates the microchannels in the stigmatic cuticle". Ruiter et al. (1997) confirmed that the proteins, which most probably play an active role in pollen-pistil interactions, are located exclusively in the

pollen coat. In a recent review, Willemse (1999) summarizes the nature of the pollen coat and its effects with respect to pistil activation. Attention is called also for the poral sporophytic proteins as published by Pacini and Juniper 1979a, b.

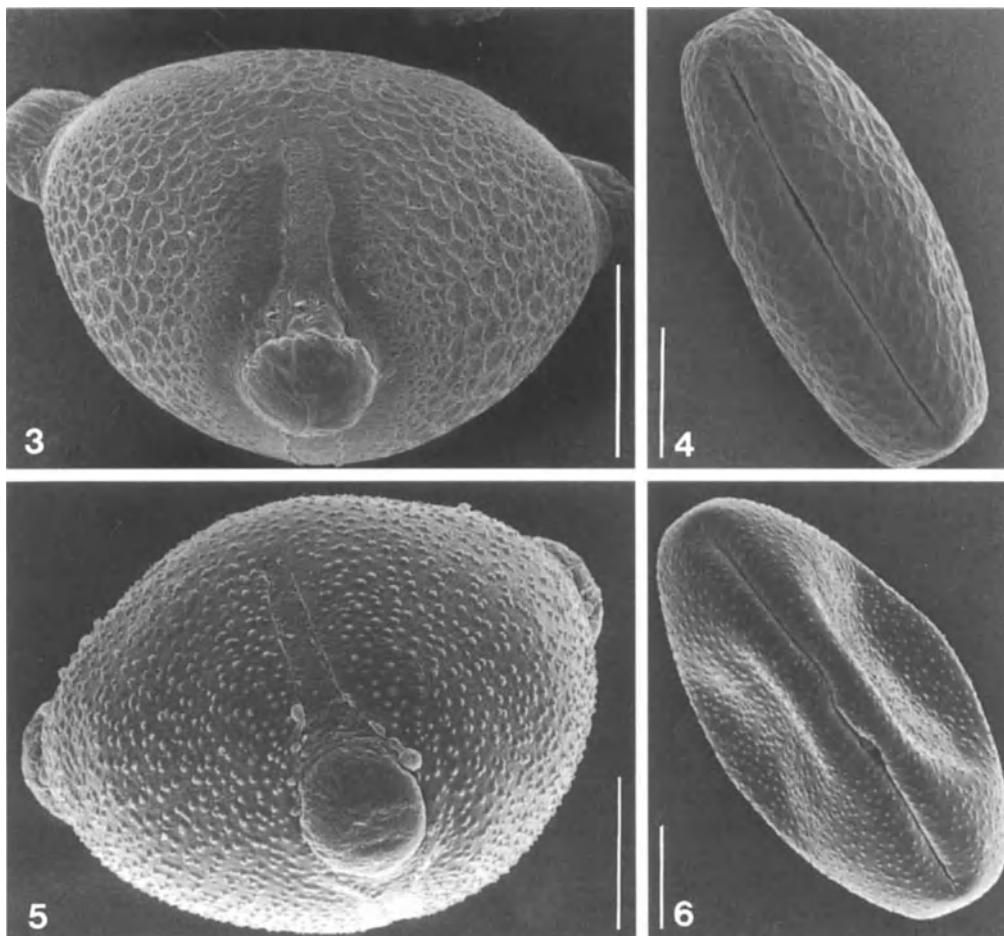
In this context an unexpected, strange phenomenon should be addressed. The inaperturate pollen grains of most of the aperigoniate Aroideae – Araceae (sensu Mayo et al. 1997) have an outer, often elaborated, specific exine layer (stratum), which is not resistant to acetolysis (Weber et al. 1998, 1999). This (polysaccharidic) layer was interpreted as being not homologous with an ektextine. It is located on top of a likewise uncommon spongy, thick endexine (Fig. 2). Whether and to what extent these striking characters are functionally correlated with the very peculiar pollination mode in the aperigoniate Araceae, remains a matter of speculation. This is inter alia apparent from the paper by Pacini and Juniper (1983): “PAS-positive spines of *Arum* could be involved in the complex *Arum* pollination system to control recognition between pollen surface and stigma”. However, this uncommon outer wall layer seems to be of high significance, as nearly all aperigoniate Aroideae show this unusual feature.

Infratectal stratum. For some general remarks the reader is referred to chapter A. As far as the presence of columellae or similar structures is concerned, attention should be drawn to the exine arcade or likewise (if present) the space between infratectal and supratectal columellae, which are acting as storage for sporophytically derived fluids or pollen coatings (e.g. Furness 1995). But they also play a role in the harmomegathic effect. An example is shown by Suarez-Cervera et al. (1995): in *Euphorbia* and *Chamaesyce* the partly unique apertural sporoderm may be an adaptive modification to harmomegathic changes and thus facilitate germination and the formation of the pollen tube. In general, the respective pollen shape in dry state is not correlated with the actual pollination syndrome. Anemophilous taxa show same

harmomegathic effects as pollen of zoophilous taxa. Yet the harmomegathic effect is, of course, related to the form and number of the apertures (see below). Similarly, the function of caveae [in Asteraceae] is linked to harmomegathy (Bolick 1978, p. 214). At first look, there is evidence that folding of apertures in dry state preserves humidity in the air-transported pollen (Blackmore and Barnes 1986). However, it is well known that apertures may not be infolded in dry state; instead, the mesocolpial areas may be infolded (The Figs. 3–6 exemplify these strategies: while in dry *Rhamnus* pollen grains the apertures are infolded, in *Cornus* the contrary is given). Whether this controversial feature also preserves humidity is unknown. The whole system of harmomegathy is a rather complex one and deserves additional investigations.

So far, there is nothing known about functional aspects of the absence of columellae as, for example, the granular infratectum in many Annonaceae (Le Thomas 1981). Generally, more investigations are necessary also in this field.

Foot-layer. The foot-layer is not always compact, as is generally shown in textbook drawings, but broken, or crossed by small tubules, the latter probably in connection with the likewise less compact endexine (see next page). Fragmentation or even the reduction or absence of a foot-layer in *Sesamothamnus* tetrads (Suarez-Cervera et al. 1992), in orchid monads, permanent tetrads or in orchid polyads: see Burns-Balogh and Hesse (1988), Hesse et al. (1989) and in many Annonaceae, respectively (e.g. Le Thomas 1981), is only on the first sight functionally significant. There are parallel developments only because of quite different pollination modes in Annonaceae and orchids. The fragmentation of the foot-layer may result also from its rupture during pollen development. It is probably related to rapid water uptake and the harmomegathic function of the sporoderm. In general, separation of exine units and not only of the foot-layer can be considered an adaptation to volume change during pollen development and/or for water uptake.



Figs. 3–6. SEMs of mature pollen grains in hydrated (3 and 5) and dry state (4 and 6), respectively. Note variability of infoldings in dry pollen grains, for details see text. 3 and 4 *Rhamnus alaternus* (Rhamnaceae), 5 and 6 *Cornus floribunda* (Cornaceae). Bars: 10 µm

Endexine

Our knowledge on endexine function, even its presence in monocots, is very meager. Attention should be drawn to a general point: at least some monocot families have indeed an endexine, which was so far overlooked or misinterpreted, e.g. in Araceae (Weber et al. 1998, 1999). Recently a special term, the “transitional endexine”, was reported for several taxa (El-Ghazaly and Rowley 1999, also for review). The authors emphasize in this connection that in considering only adult anatomies we may misread evolution. The authors stress the importance of investigating immature pollen developmental stages. However, little is known about a functional role of

the various strata during late developmental stages. Young stages of *Strelitzia* pollen show a surprisingly complex wall stratification, whereas a skin-like wall characterizes the mature grain (Kronestedt-Robards and Rowley 1989).

The endexine (and also the foot-layer) is elastic (Rowley 1990, Suarez-Cervera 1995, also for review). This is confirmed by the strikingly different outlook in many monocot pollen both in the dry and hydrated states (Halbritter and Hesse 1993; cf. the zonosulcate “hamburger-like” pollen forms of *Monstera*, *Zamioculcas*, *Gonatopus* in Araceae, Hesse et al., Grana in press). The lamellate endexine in fully zonate angiosperm pollen favors the

conspicuous shape modification between dry and hydrated pollen grains. Attention should be drawn to another, more general point: it was shown indeed that many monocots have an endexine, which had been overlooked or even misinterpreted, e.g. in Araceae (Weber et al. 1998, 1999). The endexine is generally less compact than previously thought. Many channels allow the (potential) crossings of material from the intine to the exine arcade region and vice versa (Weber 1996b, Piffanelli et al. 1998).

The harmomegathic effect depends, among other reasons, on the elasticity of the exine and the sometimes high degree of dehydration and rehydration. Pollen of some taxa does not undergo dehydration and can easily germinate inside the still closed pollen sac. This might be a natural behaviour in response to the commonly moist environment and also due to the respective pollination strategy (Pacini and Franchi 1982).

Intine

Reference is made to a classical paper by Heslop-Harrison and Heslop-Harrison (1991). To my knowledge the role of the thick intine in angiosperm apertures is not fully understood, apart from their known role in pollen germination. The presence of gametophytic proteins in the compact intine body, warrants further investigation. Similarly, further aspects of the role of a thickened intine concerning aperture configuration, pollen stability, pollen tube formation, and especially the role of the plasmalemma infolded in the tubules of thickened apertural intines should be considered. The change of membrane-associated Ca^{2+} - level and its role in pollen hydration, germination and pollen tube tip growth are important aspects, but are not within the scope of this paper (see Malhó 1999).

Apertures

As stated above, Thanikaimoni (1986) has reviewed form and function of apertures, especially in relation to pollination modes. The obvious question "why several apertures,

and not a single one" is addressed in another classical paper (Heslop-Harrison 1979, p. 826 ff). It was assumed that the ratio of apertural area, measured as mean area of aperture \times mean number per grain, to total wall area would vary less among and within the groups than pollen grain size itself, certainly among species with dry stigmas. The adaptive value of forate exine is based on allowing the highly localized activation of apertural intine sites.

Earlier, numbers and distribution of apertures were associated with an evolutionary scheme in relation to various groups of insect pollinators (e.g. Klaus 1987). This idea cannot longer be supported, because of two new findings: polyforate pollen is found in more primitive angiosperm families, e.g. in the Trimeniaceae (Sampson and Endress 1984), and polyforate pollen has existed since the Cretaceous (Ward and Doyle 1994).

Attention should, however, also be focused on sometimes hidden functional aspects, if no apertures are present at all (Furness and Rudall 1999). Inaperturate, omniaperturate, or functionally monocolpate taxa are found in many monocot, but also basal dicot families, preferring wet or moist habitats. Under moist conditions, pollen may not undergo desiccation, and no harmomegathic effect will take place. Thus, the omniaperturate condition with its reduced exine might be an adaptation to increased germination efficiency under this condition: the pollen tube is formed more quickly and can emerge from any pollen region (Furness and Rudall 1999). The following citation (Linder 1998, p. 14) explicitly addresses the problem: "the possibility that apertures might be correlated to or affected by wind pollination has not been explored before: ... however the association between anemophily and ulcerate pollen is not easy to either demonstrate or to reject".

Onci and Zwischenkörper

Although the role of a pectinic zone is well known for some taxa (Heslop-Harrison and Heslop-Harrison, 1991, also for review), some questions are still open. First, terminology is

inconsistently applied. Despite several papers in the last decade, exact definitions are still rare, as in the case of Zwischenkörper (often seen as identical with oncus, Praglowski and Raj 1979, but see in this respect El-Ghazaly 1999). Substantial confusion exists also in relation to the terms “oncus” including inner and outer oncus, and the onciform zones in various monocot pollen grains (Rowley et al. 1997). It is unclear if the onciform zones in some monocots are homologous to the Betulaceae onci. Moreover, the status and function of the onciform zones deserve further research. Secondly, it is a matter of interpretation if onci (i.e. the inner and the outer one) or a Zwischenkörper (in its meaning not identical to the various onci) are parts of the sporoderm. In this paper they are treated separately, following El-Ghazaly (1999). In the context of this paper, this problem will not be discussed at length. It is just another example of the controversial use of seemingly simple palynological terms, being applied to the biodiversity of angiosperm pollen walls. One clear statement comes from Barnes and Blackmore (1986): Onci may function during germination as a pre-formed tip to the emerging pollen tube, further in the control of rehydration during germination. Whereas onci of the grass type are also present in, for example, Asteraceae, Betulaceae, or *Eucalyptus* (Heslop-Harrison and Heslop-Harrison 1991), they are not omnipresent in all of the angiosperms.

C. Special case studies

Non-stratum-based correlations between pollen walls and several modes of pollination were reported or at least assumed, but they cannot be seen as isolated in terms, for example, of role of the tectum etc. There is pollen that lacks the classical exine stratification, exhibiting a strong exine reduction. In unrelated taxa of seagrasses, occurrence of more or less filiform pollen may be a matter of convergence. The filiform appearance may be caused by a total lack of an exine or by a skin-like exine layer only (see the various papers by

Cox, e.g. 1991, on underwater pollination). Surprisingly, the pollination mode in the various families of seagrasses with filiform structures (either filiform pollen or pollen grains packed into threadlike search vehicles, Cox and Knox 1989) is not so uniform, as is obvious from (more or less spherical) pollen grains included in “search vehicles”. Apparently some questions must be raised about well known results. Ackerman (1995) reviewed various “strategies”, and in evaluating current ecological and/or evolutionary models he stresses the consistency of the biophysical model with mathematics and nature, disfavoring the random search theory. He concludes that in Potamogetonales filiform pollen is convergent, having evolved several times in different families. Reproductive strategy does not provide sufficient information to understand this convergence. Cox and Humphries (1993) also raise the question if filiform pollen represents really an evolutionary convergence or if all such taxa descend from a common ancestor having filiform pollen. They express the interesting idea that filiform pollen in seagrasses may be seen as preadaptation for water surface pollination.

In this connection, singular threadlike pollen (30x longer than broad) of land plants as *Crossandra stenostachya* (Acanthaceae) (Brummit et al. 1980), is a puzzling finding. However, in other species, e.g. *Crossandra nilotica* (Halbritter unpubl.), the elongated pollen is not “filiform”. By contrast, pollen of *Crossandra flava* resembles the polypligate pollen type with “6 or more” colpi probably representing three real colpi and three pseudo-colpi. It is debatable if this feature leads to the evolution of various polypligate pollen. Nothing is known about pollination biology in either species, and the quite uncommon ratio of polar and equator axis is not understood.

According to Osborn and Philbrick (1994, p. 261) pollen of the obligately submersed *Callitrichia hermaphroditica* is not filiform, as might be expected, but spherical. Nevertheless, pollen is functionally “filiform”, because pollen germinates within the locule, and a tangled mass of pollen tubes results.

Philbrick and Osborn (1994, p. 378) address another ecological point: "There is also an apparent association between the growth habit (terrestrial versus amphibious) and the relative thickness of the basal layer it is not known whether the exine is actually selected against during the evolution of hydrophily or is simply lost due to genetic drift". This is interesting, but the same authors are not correct in stating on p. 371 that exine reduction is one of the few morphological features that is unique to hyphydrophilous pollination systems. Reduced exines are much more abundant in angiosperms, especially in various monocot taxa. An unsurpassed paper is Kress (1986) with a survey on "exineless" pollen and pollination. "Exineless" in this context means that at least in small parts of the pollen surface exinous (sporopollenin) remnants were present. As shown above, exine reduction in omniaperturate pollen may be an adaptation to increased germination efficiency.

Fossil pollen and climatic conditions of the past

Research on the correlation between sporoderm stratification and pollination focuses on extant taxa. The idea on analyzing fossils was never really followed, because it would require in-situ findings of pollinating insects loaded with pollen, but Crepet (1979) should not be overlooked in this context. The co-evolution of angiosperms with their pollination vectors is a very important aspect of the evolution of the plant kingdom, as was stated by Traverse (1988). An interesting idea was raised by Willemstein (1987, p. 310), concerning the age of the genus *Carex* and its pollination mode. Although the family is reported only since the Eocene, it is assumed to be much older. The presumed entomophilous predecessors must have had different pollen, not recognized today. Some papers refer to relationships between ornamentation of fossil pollen and the putative pollination mode. The conspicuous, perfectly preserved viscin threads of some fossil Rhododendroideae point towards a sophisticated pollination syn-

drome, almost identical to comparable extant taxa, but all of them without referring to sporoderm features (Zetter and Hesse 1996). However, some ideas on correlations of sacci-like features and pollination were published, and I would like to address particularly the homology question of distinct pollen or sporoderm features in this context. The fossil form-genus *Lactoripollenites* evolved a solution to meet the constraints of pollination in the early Late Cretaceous. This feature may be regarded as not more than a functionally analogue to gymnosperm sacci. Hence, there is a lack of homology in saccus-like structures in Lactoridaceae and fossil/living saccate gymnosperms. The modern analogue *Lactoris fernandeziana*, which is now endemic to Masatierra, one of the Juan Fernandez islands, was thought to have the same (animal) pollinators as the Juan Fernandez endemic *Drimys confertifolia* (MacPhail et al. 1999, Sampson 1995). But no biotic pollinators have ever been reported for *Lactoris*, and anemophily is concluded (Bernadello et al. 1999). In general, investigating TEMs of fossil pollen and trying to find correlations with pollination sounds very promising.

Pollen polymorphism

Erythronium grandiflorum exhibits a strong pollen-color dimorphism; The pollen color variants were used to measure pollen carryover and deposition. It was shown that the dispersal of red or yellow pollen has implication for the reproductive success in this species (Thomson and Stratton 1985; pp. 433–437, Thomson and Thomson 1989, pp. 657–661). The dimorphic anthers of *Lagerstroemia* (Pacini and Bellani 1986) produce blue fertile pollen and yellow feeding pollen (the colour is due to the pollenkitt!). Both sorts of pollen grains differ inter alii in exine pattern, pore number, and pollenkitt amount (the degree of surface coating, Muller 1981). Feeding pollen germinates on the stigma, but does not reach the style. It is not clear, whether dimorphic pollen is in fact correlated with heterostyly. Mejias and Diez (1993) found dimorphic pollen in various

populations of the non-heterostylous *Sonchus oleraceus*: the phenomena may reflect the chromosomal constitution in this amphidiploid species. Olsson (1974) and Radivo (1998) reported dimorphic pollen in the heterostylous *Primula vulgaris*, but failed to find a notable relation to heterostyly, because dimorphic pollen exists in both morphs.

Another striking feature is the pseudopollen reported for some Theaceae (Tsou 1997). The pseudopollen grains are produced in the connective, are released into pollen sacs, and then mix with real pollen grains. Pseudopollen show a different ornamentation and a lot of thin wall areas pretending apertures. The role of this pseudopollen in pollination is not clear, pollen and pseudopollen was found on honeybees. The author suggests that pseudopollen might fulfill the demand for food supply of flower visiting insects in a deceptive manner.

Conclusions and outlook

To conclude, there is no better statement to answer briefly the question of whether and to what extent the pollen grain ornamentation and strata are related to pollination than the realistic statement by Philbrick and Osborn (1994, p. 370): "Several studies have shown positive correlations between pollen structure and the nature of pollen vectors ... whereas others report a lack of such associations". Beside this some features, for example the oncus/Zwischenkörper question, or the role of harmomegathy in relation with our topic, deserve elucidation. Then hopefully we will have a better understanding of the complex relationship between pollen stratification (and also ornamentation) and pollination.

Material and methods

The plant material for the micrographs was obtained from the Botanic Gardens Munich and Vienna, respectively: *Argyranthemum frutescens* (Asteraceae), *Pistia stratiotes* (Araceae), *Rhamnus alaternus* (Rhamnaceae), and *Cornus floribunda* (Cornaceae). The pollen material was prepared

for TEM and SEM according to Weber et al. (1998).

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From anther and pollen ripening to pollen presentation

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Abstract. The events and processes occurring between pollen maturation, opening of the anther and presentation of pollen to dispersing agents are described. In the final phases of pollen development, starch is always stored; this occurs before the anther opens. Depending on the species, this starch may be totally or partially transformed into: (a) other types of polysaccharides (fructans and rarely callose); (b) disaccharides (sucrose); (c) monosaccharides (glucose and fructose, all situated in the cytoplasm. While awaiting dispersing agents and during dispersal, polysaccharides, especially fructans, and sucrose may be interconverted to control osmotic pressure and prevent loss and uptake of water. Opening of the anther is preceded by disappearance of the locular fluid and in many cases by partial dehydration of the pollen. Pollen generally has a water content between 5 and 50%. Pollen with a high water content may or may not be able to control water retention during pollen exposure and dispersal. Pollen may be dispersed in monads or grouped in pollen dispersing units by the following mechanisms: (i). tangling of filamentous pollen; (ii). adhesion by viscous substances (pollenkitt, tryphine, elastoviscin) derived from the tapetum; (iii). common walls. When the anther opens, the pollen may be dispersed immediately, remain until dispersed (primary presentation), or be presented to pollinators in another part of the flower (secondary presentation).

Key words: Anther, pollen, dispersing units, dehydration state, carbohydrate reserves, pollen presentation.

The reproductive structures of animals usually develop in elongated cavities open at one end; at maturity they emerge from the end into the outside world. In plants, the reproductive structures develop in closed cavities which open at maturity to enable dispersal. During development, spores and pollen are immersed in a liquid that conveys nutrients from the tapetum, that is, from the innermost part of the sporophyte (Pacini and Franchi 1992) to developing spores or pollen. When the pollen is almost ripe, the tapetum disappears, having fulfilled its function. The locular fluid remains for a short period of time (Pacini 1997) and then disappears enabling pollen dispersal at anther opening. The pollen itself normally loses water just before and/or just after the anther opens; in this way it becomes dormant thus resisting the stresses of the external environment (Fig. 1, Table 1).

There appears to be a progressive reduction in water content from the spores of early land plants to gymnosperm pollen. Similarly spores of early land plants have chloroplasts whereas angiosperm pollen has proplastids or amyloplasts. At dispersal, the spores of Bryophytes often have chloroplasts, vacuoles and a high water content (Clarke 1979); Pteridophytes may have spores with: (a). proplastids or amyloplasts, a low water content (about 5%) and no vacuoles; (b). chloroplasts as the spores of *Equisetum* and *Matteuccia* have (Cran 1979,

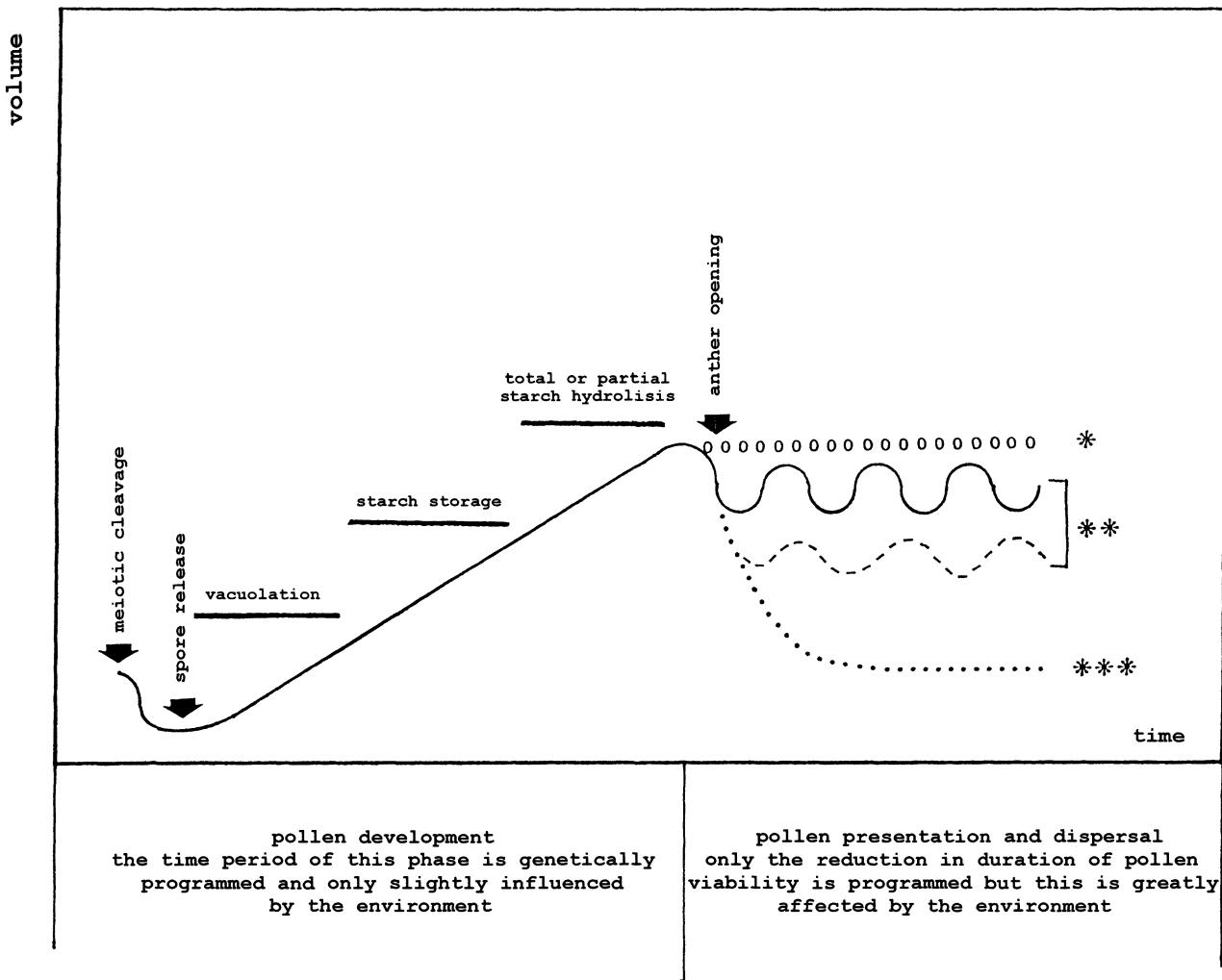


Fig. 1. Graph showing changes in volume of pollen grains during development, presentation to dispersing agents, and dispersal. A decrease in volume occurs after release of the microspores; this is followed by a constant increase proportional to vacuole formation. Vacuole formation is followed by storage of starch by plastids; depending on the species, vacuoles may form once or twice. Just before the anther opens, all or part of the starch may be hydrolysed, depending on the water content at dispersal and whether water retention mechanisms exist. Depending on the capacity of pollen to retain water, the following categories can be identified: ○○○○○○ pollen that is not dehydrated when the anther opens and is dispersed in the sea; submarine pollination; ——— pollen that has completed the process of dehydration prior to anther opening; anemophilous and entomophilous pollination; - - - - - pollen that has not completed the process of dehydration when the anther opens but completes it during exposure or dispersal; anemophilous and entomophilous pollination; ••••• pollen that is not dehydrated when the anther opens and that has no mechanism for retaining water; anemophilous pollination and entomophilous pollination. *The pollen remains viable for as long as basal metabolism continues. **The pollen remains viable under suitable environmental conditions for as long as cytoplasmic carbohydrates protect it against water loss. ***The pollen loses water and dies under environmental conditions of low relative humidity and high temperature. Modified from Pacini (1990)

Lehman et al. 1984). The metabolism of spores with chloroplasts, vacuoles and high water content is therefore still active at dispersal,

whereas spores with amyloplasts and low water content have a slow metabolism and develop a type of resistance similar to

dormancy in seeds. At dispersal, pollen of all gymnosperms and most angiosperms is partially dehydrated, that is, with a water content of less than 20%, and chloroplasts are absent (Pacini 1994).

The aim of this review is to describe all the processes occurring between pollen maturation and dispersal, thus bringing together topics that have always been considered separately because of their origin in different disciplines. Specifically, an attempt will be made to link the aspects of cell differentiation of pollen to the ecological aspects of pollination.

Anther structure

Anthers may be found in uni- and bisexual flowers, and except in a few cases (see Ackerman, this volume), they range in shape from spherical to prismatic, with different degrees of elongation. They may contain two or more locules; the most frequent number, however, is four, each anther with two apertures called stomia (sing. stomium) (Endress 1996). In *Ricinus communis* and *Parietaria judaica*, which have explosive dehiscence of the anther and pollen expulsion, there are only two locules and one stomium (Bianchini and Pacini 1996, Franchi unpublished data). This alternative structure may be due to the fact that simultaneous expulsion by two stomia would be impossible or ineffective.

Stamen and anther structure varies from species to species and between different stages of development. In most cases, the mature anther contains epidermal, endothelial, middle layer and connective cells (D'Arcy and Keating 1996).

The epidermis may have stomata, especially near connective tissue. In many cases of anemophilous pollination, this is the only cell layer that persists in the mature anther. In this situation its functions as the mechanical layer (Bianchini and Pacini 1996). If other cell layers of the anther persist, the mechanical layer may be found elsewhere. The endothecium, consisting of dead cells with sometimes lignified thickenings, is the mechanical layer involved

in opening most anthers. It is absent in marine monocots (McConchie and Knox 1989).

Depending on the type of pollination, pollenkitt, orbicules, and the peritapetal membrane may remain (Pacini 1997). Connective tissue joins the four lobes that comprise most anthers, and it contains the vascular bundle.

The pollen develops in a cavity known as the loculus; if the tapetum is of the parietal type (i.e. secretory), there is a space containing locular fluid between the tapetal cells and the developing pollen grains. If the tapetum is of the amoeboid type, it adheres to the pollen and the locular fluid only becomes abundant after the tapetum has disappeared (Pacini 1997).

Locular fluid conveys nutrients from the sporophyte to the developing male gametophyte; thus its composition changes in the different stages of development, and the concentrations of dissolved substances are reduced drastically as the pollen matures (Clement et al. 1998).

The anthers are carried by filaments which may be erect, curved or pendant in the mature flower depending on the type of pollination. The main task of the filaments is most likely to favour presentation and release of pollen. In some cases (e.g. the genus *Cucurbita*) filaments are lacking and the anthers are massed together (Nepi and Pacini 1993).

The number of stamens per flower varies from one, as in most Asclepiadaceae and Orchidaceae, to several hundred, as in certain Myrtaceae and Cistaceae (Endress 1994). When there are many stamens, pollen presentation may be simultaneous, as in most Myrtaceae (Lughadha and Proenca 1996), or they may ripen one at a time, as in *Helleborus* (Vesprini and Pacini in press), where the number of anthers opening each day is temperature dependent. The higher the temperature, the more anthers open each day in this species and the shorter the life of the flower.

Anther form and size vary widely. This diversity may depend on the type of pollen dispersing unit, the pollen/ovule ratio, the pollen vector, and if the latter is an insect, the path it is obliged to follow in visiting a

Table 1. Diagram showing the formation and types of pollen carbohydrates and their ecophysiological significance during pollen exposure and dispersal. The mean life of the pollen grain and the time of pollen tube emission are influenced by the water content and the type of carbohydrates present. Some fructans, being different from starch because soluble, are responsible for part of the osmotic pressure; besides, all types of fructans being soluble or insoluble are easily polymerisable and depolymerisable, and thus are able to vary the osmotic pressure according to the environmental conditions. Fluctuations in temperature and a decrease in relative humidity results in an increase in osmotic pressure which in turn hinders water loss and ice formation within the pollen grain. Vice versa, an increase in relative humidity induces a decrease in the osmotic pressure. Pollen grains with a water content of less than 30% have a furrow which allows dehydration and rehydration of the pollen grain; those with a water content higher than 30% are devoid of furrows

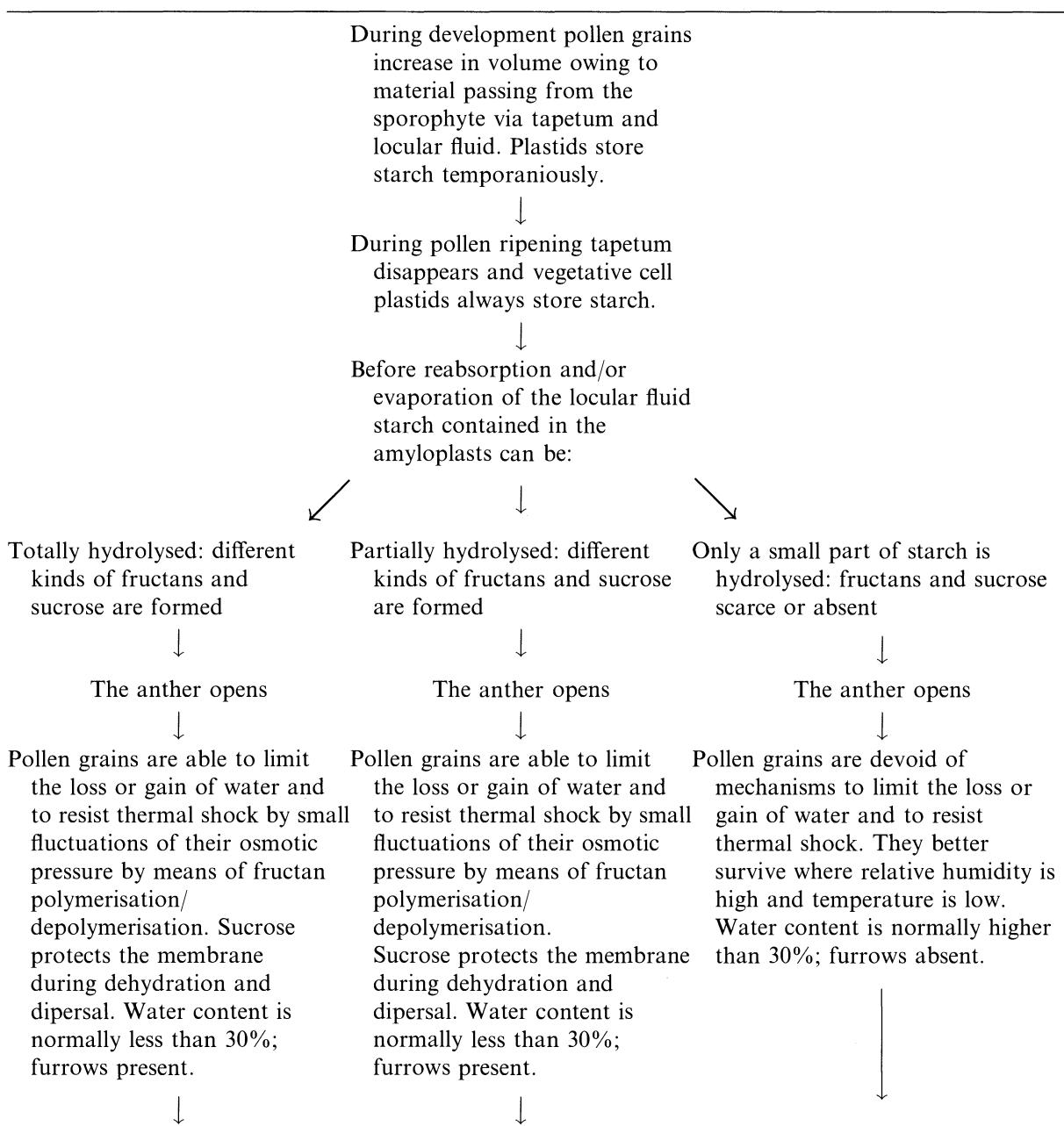


Table 1 (continued)

↓ Pollen grains are able to self-regulate. They can survive different environments and fluctuations of relative humidity and temperature. Their mean life lasts a few days. Pollen tube emitted one hour or more after landing on the stigma.	↓ Pollen grains are able to self-regulate. They can survive in different environments and fluctuations of relative humidity and temperature. Their mean life lasts a few days. Pollen tube emitted one hour or more after landing on the stigma.	↓ Pollen grains are devoid of autoregulation mechanisms and survive in environments where relative humidity is high and temperature is low. Their mean life lasts only a few hours. Pollen tube is emitted a few seconds/minutes after landing on the stigma.
Examples: <i>Lycopersicum peruvianum</i> , <i>Lilium</i> sp.	Examples: <i>Helleborus boreocnei</i> , <i>Malus domestica</i>	Examples: <i>Cucurbita pepo</i> , <i>Parietaria judaica</i> , <i>Zea mays</i>

flower. For a more complete discussion of these topics see Endress (1996) in the volume edited by D'Arcy and Keating (1996) on the various aspects of the anther.

Pollen and anther maturation

Pollen development draws on substances wholly or partly derived from these sources: (a). Photosynthesis by the mother plant, especially in plants flowering in the presence of leaves. (b). Photosynthesis by the anther, especially when the calyx and corolla are absent or reduced. (c). Material stored in other parts of the flower or plant, especially in plants that flower before they develop leaves. (d). Utilization of transient cells and material of the anther, such as the tapetum, the connective tissue, the middle layer and the callosic walls (Pacini and Franchi 1983, Clement and Audran 1999).

Cellular components of pollen

Although angiosperm pollen has a high diversity, it is characterized by certain common features: (a). It contains carbohydrate reserves for gametophyte development and its maintenance up to germination (Table 1). (b). It has two walls: the exine and intine (see also Hesse, the sporoderm, this volume). (c). It contains the gametes or their precursors.

Practically all pollen contains carbohydrate reserves and has two walls. Exceptions are marine monocots which have filamentous pollen without exine (McConchie and Knox 1989),

and certain land plants, such as Musaceae and Heliconiaceae that grow in environments with a high relative humidity. This genus has pollen devoid of or with reduced exine (Kress 1986). Pollen dispersed with a high water content germinates within a few minutes after arrival at the stigma (*Cucurbita pepo*, *Lavatera arborea*). Pollen of these species has three walls: exine, intine and a thin callosic wall (Table 2). They also have cytoplasmic reserves of callose (Nepi et al. 1995, Nepi and Pacini 1999).

At dispersal, gymnosperm pollen is composed of from one to more than ten cells (Pacini et al. 1999). In contrast in angiosperm pollen consists of 2–3 cells being generative and sperm cells. These cells are fusiform with a strong cytoskeleton, which perhaps serves to penetrate the pollen tube (Tanaka 1993) and travel inside it (Heslop-Harrison 1987). However, there are some exceptions. The generative cells of ripe pollen of Orchidaceae (Pandolfi et al. 1993, Pandolfi and Pacini 1995) and the genus *Quercus* (Barni unpublished data) are roundish (Fig. 2e). A likely explanation for these exceptions is that in these two groups, pollen tubes are emitted more than 24 h after the pollen reaches the stigma. To maintain the fusiform shape of the generative and sperm cells, the cytoskeleton must be kept active, which requires energy. Therefore, perhaps to conserve this energy, the generative cell remains spherical only becoming fusiform when it is about to enter the pollen tube. This also is the case in species that have pollen that must

Table 2. The main types of carbohydrates present and % water content of pollen. Starch coexists with fructans as in *Opuntia dillenii*, or it can be the only polysaccharide reserve as in *Cucurbita pepo*; fructans also can be the only carbohydrate reserve as in *Helleborus boconei*, *Cucurbita pepo* and *Zea mays* have the highest % water content, callosic cytoplasmic reserves as well as a callosic wall beneath the intine at dispersal (1), in addition their pollen germinates few minutes/seconds after landing on the stigma. Data from Franchi et al. (1997) and unpublished data. ND Not available data

	Starch (IKI test)	Callosic cytoplasmic reserves (1) (aniline blue test)	Fructans (inulin test)	Sucrose ($\mu\text{g}/\text{mg}$)	Glucose ($\mu\text{g}/\text{mg}$)	Fructose ($\mu\text{g}/\text{mg}$)	Water %
DICOTS							
Cactaceae							
<i>Opuntia dillenii</i> (Ker-Gawl.)	+	ND	+	187.1	62.1	32.6	ND
Cucurbitaceae			-	1.9	11.7	12.8	44.6 ± 0.9
<i>Cucurbita pepo</i> L.	+	+					
Malvaceae			+	43.0	7.7	14.1	ND
<i>Alcea rosea</i> L.	+	+					22.7 ± 2.1
Oenotheraceae							
<i>Oenothera organensis</i> Munz.	?	+		140.8	12.0	3.3	
Passifloraceae			+				
<i>Passiflora caerulea</i> L.	+	-		43.8	7.7	14.1	
Ranunculaceae							
<i>Helleborus boconei</i> Ten.	-	-		70.2	1.6	1.2	ND
Urticaceae							
<i>Parietaria judaica</i>	+	-	-	ND	ND	ND	ND
MONOCOTS							
Poaceae							
<i>Sorghum bicolor</i> L. (A2112)	+	+	+	81.8	4.6	5.0	ND
<i>Zea mays</i> L. (W229)	+	+	+	60.9	4.9	4.9	35.6 + 0.2
Orchidaceae							
<i>Stanhopaea oculata</i> (Lodd.)	-	-	+	95.6	0.1	0.2	ND
Palmae							
<i>Chamaerops humilis</i> L.	-	-	+	132.3	0.7	0.7	15.3 ± 9

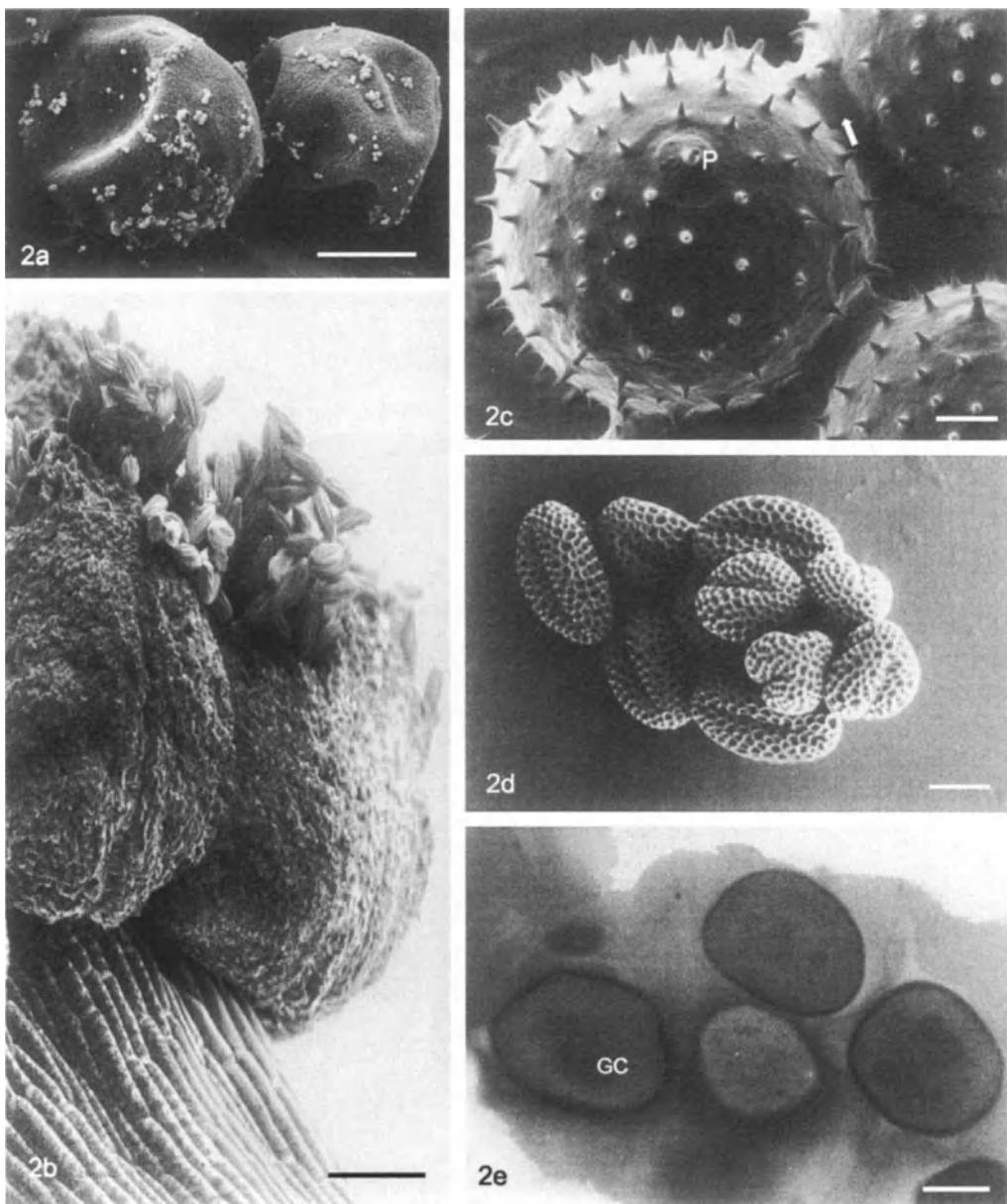


Fig. 2. Different types of pollen dispersing units in gymnosperms and angiosperms. **a** Two pollen grains of *Cupressus sempervirens* (Cupressaceae): note deflated football shape and single and grouped orbicules on the surface. Bar: 25 µm. **b** A pollen sac of *Ephedra americana* (Ephedraceae) attached to the filament at the time of pollen exposure. Clumps of pollen are visible near pores. Bar: 200 µm. **c** Pollen in a mature anther of *Cucurbita pepo* (Cucurbitaceae). The pollen has a thick layer of pollenkitt over the pore region (*p*) and microsculpture between the spines. The pollenkitt holds the pollen grains together (arrows) and to the anther. Bar: 20 µm. **d** A clump of pollen of *Brassica oleracea* held together by tryphine. Despite the tryphine, the exine microsculpture is visible. Bar: 20 µm. **e** Pollen of *Paphiopedilum villosum* (Orchidaceae) held together with elastoviscin. The pollen is binucleate and the generative cell is roundish (*GC*). Section embedded in LR white and stained with toluidine blue O. Bar: 15 µm. **f** Pollen of *Fuchsia magellanica* (Onagraceae) still on the anther. The pollen is held together and to the anther by a tangle of viscin threads attached to the pore region. Bar: 50 µm. **g** Tetrads of *Typha latifolia* (Typhaceae). The four pollen grains of the tetrad are held together by exine bridges and are not joined in the central region. Bar: 15 µm. **h** An open anther of *Acacia dealbata* (Mimosaceae) with three polyads consisting of 16 pollen grains each. Bar: 15 µm. **i** The androecium of the orchid *Ophrys insectifera* with two soft pollinia, consisting of a few dozens of massulae, partly enclosed in the anther; note two viscidia at the base of the pollinia. Bar: 150 µm

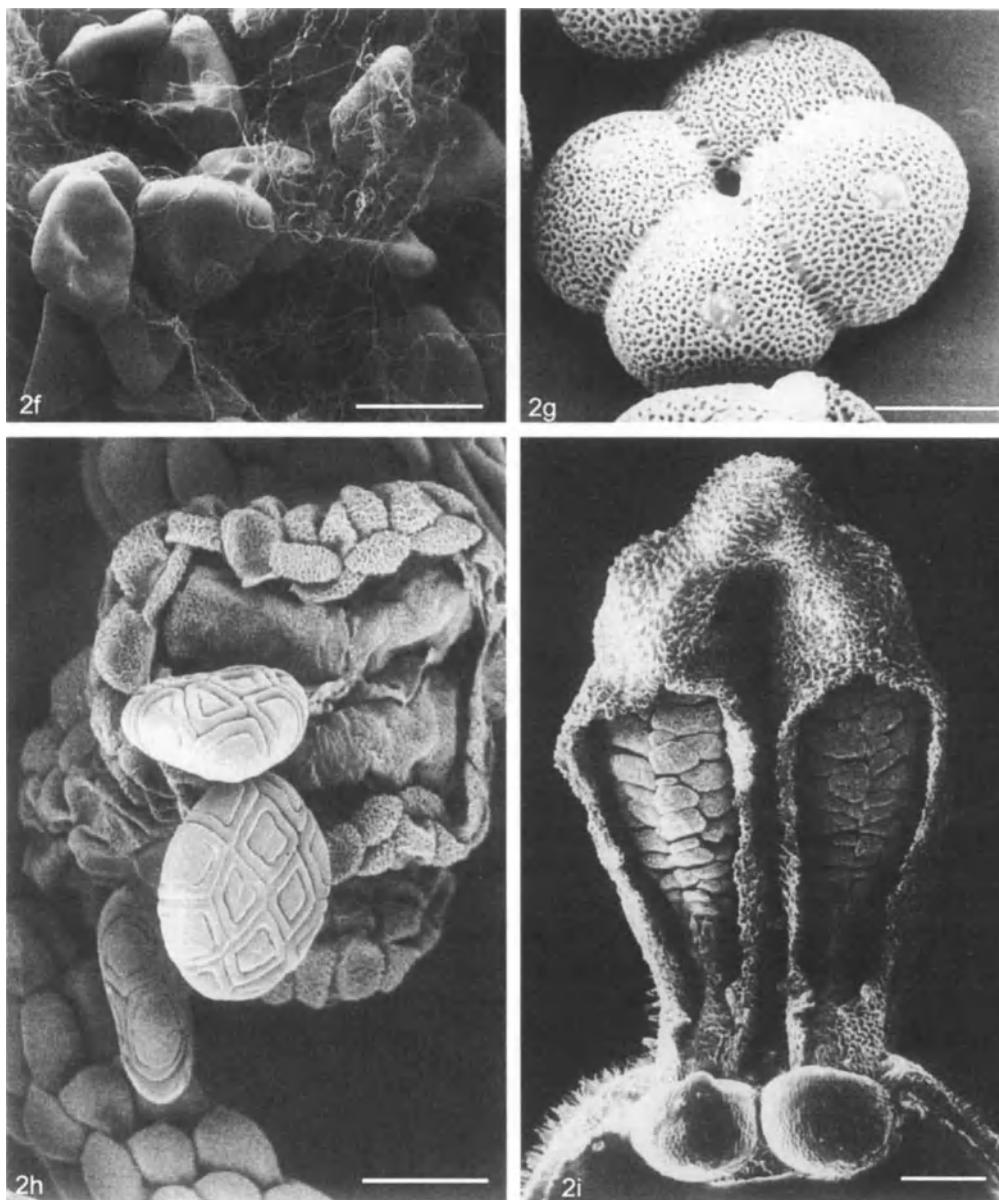


Fig. 2 (continued)

await pollinators for long periods (orchids) or that take hours to germinate (Pandolfi and Pacini 1995).

The vegetative cell also has a cytoskeleton that is usually deactivated in mature pollen but becomes active again as soon as the pollen rehydrates (Heslop-Harrison and Heslop-Harrison 1992a). In the case of certain Poaceae, such as *Triticum*, in which the pollen dehydrates only slightly, the cytoskeleton binding the plastids is not deactivated to the extent that

the amyloplasts move after anther opening and dispersal (Heslop-Harrison and Heslop-Harrison 1992b).

The tapetum in relation to pollen dispersal

The cells of the tapetum are apoptotic and supply nutrients to the developing pollen. In many cases, they also play a role in pollen dispersal. This role will be discussed here, for other functions of the tapetum, see Pacini (1997).

From the moment that microspores are released from the tetrads, the tapetum produces exine precursors, and in the case of secretory tapetum, orbicule precursors are also produced (Fig. 2a, h). The latter are involved in pollen dispersal (Keijzer 1987b). If the pollen has viscin threads of sporopollenin on its surface (Fig. 2f), the tapetum also secretes their precursors. When the exine has been formed, the tapetum degenerates and may produce viscous substances that cause the pollen to clump together. These substances are: (a). Tryphine, known in Brassicaceae (Fig. 2d) but probably present in other groups as well. Pollenkitt, which is the adhesive agent produced in most monocot (Pacini and Franchi 2000 in press) and dicot pollen (Fig. 2c). (c). Elastoviscin, which is the most viscous of the three but is only found in Orchidaceae that possess monad pollen (Fig. 2e).

Pollenkitt, tryphine, elastoviscin and viscin threads have many functions: (a). Keeping the pollen in the anther until dispersal. (b). Forming pollen clumps. (c). Sticking pollen to insects (Fig. 2b-f; see also Hesse and Vogel, this volume).

When mature pollen is observed by scanning electron microscopy, tryphine and pollenkitt may appear identical although pollenkitt is usually more abundant and may cover the exine microsculpture (Fig. 2c). Tryphine and pollenkitt however, have different ontogenesis (Pacini 1997). In both cases, the plastids of the tapetal cells become elaioplasts. In the case of tryphine, however, the plasma membrane of the tapetum ruptures as the pollen ripens, and the cell contents spill onto the pollen, where they degenerate into a more or less homogeneous fluid (Dickinson and Lewis 1973, Piffanelli et al. 1998). In the case of pollenkitt, the whole process of degeneration occurs inside the tapetal cells, clearly visible in case of male sterility (Keijzer and Cresti 1987). Prior to fusion of the locules, the pollenkitt is present as one or more spheres per cell (Keijzer 1987b, Pacini and Keijzer 1989); these spheres normally contain a homogeneous material. Pacini and Franchi (1992) denoted tapetal degeneration as “*extra situm*” in the

case of tryphine, and “*in situ*” in the case of pollenkitt as in the latter case, the tapetal cells retain their identity until the end. The pollenkitt is deposited onto the pollen when the contiguous loculi fuse (Weber 1991, Nepi and Pacini 1993).

Pollen reserves

Immediately after release from the tetrads microspores have small vacuoles, plastids store starch. In the further development vacuoles enlarge on one or two occasions, depending on the species (Pacini 1994). In the first case, vacuole formation reaches its peak during the two cell-stage; in the second case, vacuoles form during the middle microspore and two-cell stages (Pacini 1994). As vacuoles form, the pollen grains increase in volume (Pandolfi et al. 1993). This is primarily due to the storage of carbohydrate reserves, particularly starch (Pacini and Viegi 1995); the total protein content remaining constant (Pacini unpublished data). During pollen development and before starch storage, the plastids divide (Nepi et al. 1996, Pacini 1996a). After which they store one or more small grains of starch. In a few families, such as the Liliaceae (Keijzer and Willemse 1988), Magnoliaceae and Lauraceae (our unpublished data), the amyloplasts contain a very large number of tiny starch grains separated by abundant stroma. Before anther opening starch may be: completely, partially or hardly hydrolysed (Table 1).

The cytoplasm of mature pollen may contain the following carbohydrates: (a). Polysaccharides: starch (amyloplasts), fructans (vesicles or dissolved in cytosol) and callose (vesicles) (Table 2); (b). Oligosaccharides: sucrose. (c). Monosaccharides such as glucose and fructose (Table 2). Starch and fructans stain by PAS (light microscopy) and PATAG for electron microscopy. Callose can be identified by UV microscopy after staining with aniline blue (O'Brien and McCully 1981). Sucrose, glucose and fructose can be detected mainly by biochemical techniques. The presence of a, b and c varies; Table 2 shows some

examples. Not all polysaccharides present in pollen act as energy reserves. For example the callose found in vesicles within the pollen cytoplasm of *Alcea rosea*, *Cucurbita pepo* and certain grasses is not an energy reserve but rather a precursor of the inner callosic wall of the pollen tube. In these species the pollen tube is emitted minutes after reaching the stigma, whereas in species without callose, in which the water content is lower, the tube is not emitted for at least one hour (Nepi and Pacini 1999).

Mature pollen grains lack vacuoles with electron transparent contents in all except two cases. The first is the pollen of marine monocots (McConchie and Knox 1989), and the second that of the pollinia of orchids. In the latter case, the vacuoles are roundish and are derived from the breakdown of small areas of cytoplasm. Such vacuoles have been observed also in developing and mature pollen (our unpublished data) and are documented in the plates of Wolter and Schill (1986) in certain species of orchids, but not described in their text. Vesicles whose electron transparent content contains callose (Nepi et al. 1995), or those with an electron opaque content containing fructans (unpublished data) are also found in tiny vacuoles.

Lipid reserves are present in spherosomes and formed in the cytoplasm of mature and developing pollen. In our experience, these reserves are more common in pollen containing fructans rather than starch, as a polysaccharide reserve. According to Endress (1994), lipids are more common in large (80–200 µm) than in small (15–80 µm) pollen (Baker and Baker 1982).

The type of carbohydrate reserve present in the pollen grains appears to influence how much of the pollen is collected by bees. Franchi et al. (1996) determined that in about 900 species where carbohydrates reserve was detected by histochemical methods, bees actively collected these species with starchless pollen.

Partial dehydration of pollen

Pollen shape changes during development, dispersal and arrival on the stigma. Such changes are due to loss and uptake of water,

a phenomenon termed harmomegathy by Wodehouse (1935). For example most pollen is round in shape during development but becomes oval-shaped at dispersal due to water loss and folding along the furrows that are present on the pollen grain. Relating to water capacity pollen geometry and the position, form, structure and the number of furrows or pores present are more important than the cytoplasmic characteristics. These features determine how much water may be lost or taken up by the pollen grain (Pacini 1990).

At the time of exposure, pollen may contain from 1% (*Dasylirion acrotrichum*, Liliaceae) to 70% (*Humulus japonicum*, Urticaceae and *Hedychium coccineum*, Zingiberaceae) water in its cytoplasm (our unpublished data). As a rule, the higher the water content, the more active the metabolism of the pollen. However, due to its small size, pollen may lose water and decrease in volume while awaiting or during dispersal, unless the species has mechanisms to prevent this from occurring (Fig. 1). For example, some pollen contain carbohydrates that help to keep pollen vital for longer: sucrose prevents the membranes from denaturing during dehydration (Hoekstra et al. 1992, Speranza et al. 1997), and fructans vary the osmotic potential of the pollen grain, thus limiting the loss and uptake of water. Both sucrose and fructans are stored in cytoplasmic vesicles or dissolved in the cytosol and thus are more easily depolymerized than starch (unpublished data). Pollen water content at dispersal and the type and quantity of carbohydrates present are related parameters (Table 1). For example, the pollen of *Mercurialis annua* has only fructans present as a polysaccharide reserve (Table 2). When the anther opens the volume of the pollen decreases (Fig. 1) and then fluctuates with temperature and humidity (Lisci et al. 1994), yet it remains viable during this period (Pacini et al. 1997). It appears that the presence of fructans contributes to pollen longevity.

In conclusion, when the anther opens pollen may behave in different ways: (a). No dehydration, the plant grows completely im-

mersed in water, such as many seaweeds. (b). Partial dehydration prior to anther opening followed by self-regulation of water loss and uptake, e.g. *Chamaerops humilis*. (c). Partial dehydration completed after anther opening also followed by water loss and uptake, e.g. *Mercurialis annua*. (d). No adaptations to prevent water loss, plants restricted to environments or times of high relative humidity, e.g. *Cucurbita pepo*, *Parietaria diffusa*, *Zea mays* and many grasses. (Fig. 1). The third and fourth types always have fructans as a reserve; the second type has few or no fructans (unpublished data); no data is available on the first type.

The decrease in pollen water content and attainment of a type of dormancy depends on morphological factors and physiological/biochemical factors, each operating at different times. The morphological factors incorporate pollen structure (walls, furrows, pores) and the physiological factors, i.e. the type of carbohydrate reserves. Pores and furrows are evident at the tetrad stage. The various types of carbohydrate reserves can only be determined when starch hydrolysis begins prior to opening of the anther (Speranza et al. 1997, our unpublished data). Our studies have revealed the importance of certain carbohydrates in ensuring pollen survival under conditions of stress; however, there is evidence to suggest that proteins form in pollen and seeds as a consequence of dehydration. It is believed that these proteins, dehydrin or LEA (late embryogenesis abundant), help pollen to survive mainly heat but also water stress (Campbell and Close 1997).

Pollen with a high water content and few or no fructans may be dispersed by wind or insects (See Dafni and Firmage this volume). In either case, there are strategies for conveying it to the stigma within a few seconds after release from the anther. Anemophilous plants which often have pollen of this type are Poaceae. The flight of pollen of annual species takes a few seconds and covers distances of a few centimetres. The flight of pollen of perennials takes longer and covers greater distances;

in order to limit disadvantages of pollination failure, the plant has the option of vegetative reproduction. Entomophilous species with pollen of high water content either: (a). Flower at night when relative humidity is higher (*Mirabilis jalapa*). (b). Grow in the tropics or other environments with high relative humidity (*Heliconia* sp.). (c). Offer irresistible rewards that induce pollinators to visit the flower as soon as it opens as in *Cucurbita* (Nepi and Pacini 1993).

Opening of the anther

Opening of the anther is preceded by fusion of two adjacent loculi, which is caused either by opening of the septum that separates them and by rupture of the peritapetal membrane (Keijzer 1987a, Bonner and Dickinson 1989). During this process the area between the pollen grains increases suddenly, creating more space for the locular fluid, which then begins to rarefy. It is during this period that pollenkitt is deposited on the pollen grain (Keijzer 1987b, Nepi and Pacini 1993, Weber 1991).

The locular fluid may disappear by evaporation, reabsorption, or both, depending on the environmental conditions (Pacini 1994, Keijzer 1999). Evaporation is unlikely if the anther opens in an environment of high relative humidity, such as in the tropics or when the temperature is low. In *Mercurialis annua*, a species that flowers throughout the year in certain parts of Italy, both mechanisms occur. As an adaptation to these variable conditions the anthers of this species do not always open at the same time of day, as is typical in the case of reabsorption. For example, in February the anthers open around noon whereas in July and August they open around 7 am, i.e., earlier in the day when relative humidity is low and temperature high (Lisci et al. 1994). Both mechanisms have also been identified in *Ricinus communis* (Bianchini and Pacini 1996). Reabsorption, however, is programmed and regulated by the plant, whereas evaporation is not unless the plant has specific

mechanisms for limiting it. Heslop-Harrison et al. (1987) used a method to demonstrate the process of reabsorption. These authors ligated a stamen filament of *Lilium sp.* and found that the anther failed to open.

In the case of sub-marine pollination, the locular fluid is not reabsorbed (McConchie and Knox 1989). In the case of pollen that dehydrates only slightly (i.e. water content >30%), as in the Poaceae and *Cucurbita pepo* (Heslop-Harrison and Heslop-Harrison 1992b, Nepi and Pacini 1993), the locular fluid disappears and the process is arrested as the pollen can not dehydrate further. In the case of pollen that is partially dehydrated (i.e. water content <30%), as in all gymnosperms and many angiosperms, the process continues until the anther opens or just afterward (Fig. 1). In the case of cleistogamic flowers, the flowers and anthers do not open and the locular fluid is not reabsorbed (Table 4); instead the pollen tubes pierce the anther and penetrate the nearby stigma (Lord 1981).

In cases when dehydration of the anther is delayed, the pollen may germinate within the anther as occurs in some Araceae and Compositae, however, the percentage of germination varies from species to species (Pacini and Franchi 1982). Such germination occurs in pollen which is partially dehydrated at dispersal, such as *Citrus limon*, *Malus domestica* and *Olea europaea*, and for species that have only slightly dehydrated pollen, such as *Arum italicum* and *Philodendron sp.* (Pacini and Franchi 1982). In these latter two species this phenomenon is common, with up to 50% of the pollen germinating in the anther.

In other cases there are species whose anthers open before the flower, thus reabsorption is likely, and in contrast, there are those whose anthers open after the flower (Table 4), such as in *Lilium*. In this genus the anthers have stomata through which most of the evaporation of the anther fluid occurs (Clement and Audran 1995).

Opening of the anther is a process that incorporates many events that occur in the anther, flower, and external environment. No

single event or process has been identified as solely responsible for the release of pollen, instead many tissues appear to be involved in this programmed development (Bonner and Dickinson 1989). Some events, such as the formation of thickened walls in the mechanical layer, occur well before anther opening, whereas others occur in succession or just before anther opening. Table 3 shows factors known to be related to anther opening.

The mechanical layer may consist of one or more layers of cells situated in different parts of the anther, depending on the species. It is comprised of cells that often are dead when the pollen is mature, and these cells may have lignified thickenings of different functional types within the same anther. The process of cell differentiation that leads to the formation of the mechanical layer often begins during tapetal degeneration (Keijzer 1999). As a result of the drying of all, or part of the anther, and in relation to the disposition of the thickened areas, the cells of the mechanical layer change their shape, causing stretching and folding. This action leads to opening of the anther and pollen presentation or expulsion (Manning 1996, Bianchini and Pacini 1996).

The mechanical layer of *Parietaria judaica* is not situated in the anther but in the filament. When the anther is mature, i.e. thoroughly dry, the filament bends suddenly, opening the anther and launching the pollen (Franchi, personal communication). In *Ricinus communis* the anther has a mechanical layer with different cell types that differentiate according to the form and disposition of the thickenings (Bianchini and Pacini 1996). As only certain areas have a cuticle, transpiration is not uniform over the anther surface. Taken together, these features lead to explosive opening of the anther and expulsion of the pollen. In this case, the distance the pollen is launched is inversely proportional to the relative humidity; the lower the relative humidity, the further the pollen is launched. In *Ricinus*, *Parietaria*, and all other cases of anemophilous plants that expel their pollen, the mechanism appears to function in the pollen becoming airborne. Pollen is also

Table 3. Main types of structures/devices and environmental parameters responsible for anther dehydration and opening. Where pollen grains are only slightly dehydrated at dispersal and they lack mechanisms to retain water only locular fluid resorption/evaporation occurs

Structure/devices and events involved	Structure/device type and manner of involvement	References
Mechanical layer	It is formed by cells with wall thickenings containing lignins. This layer is more commonly present around the loculus with the exception of the stomium area. When the anther loses water these wall thickenings are responsible for the change of volume and cell shape and this determines anther opening as well as exothecium upset. When exothecium upset is sudden, pollen is launched.	Foster and Gifford 1974, Noel 1983, Bianchini and Pacini 1996
Vascular bundle	Phloem sap influx ceases because pollen is mature	Pacini et al. 1986
Anther stomata	Especially when the stomata open later than the flower, anther water loss may occur via evaporation and transpiration; when stomata are experimentally sealed off the anther does not open.	Keijzer et al. 1987
Cuticle, often undulated to increase the external transpiring surface	The presence of a thick cuticle in some regions of the anther induces evapo-transpiration in other parts where the cuticle is reduced or absent.	Keijzer 1987a, Keijzer et al. 1987, Lisci et al. 1994, Bianchini and Pacini 1996
Fusion of contiguous loculi	This process causes a sudden increase in volume of the cavity where the pollen grains are located without an increase in locular fluid volume. This step marks the start of pollen dehydration.	Bonner and Dickinson 1989, Nepi and Pacini 1993, Keijzer et al. 1996
Pigments in the epidermis and in the endothecium	These pigments are believed to absorb light energy and to induce evaporation.	Hanning 1910
Septum degrading enzymes	These enzymes are responsible for the detachment of the septum from the stomium area, as a consequence contiguous loculi fuse.	Keijzer et al. 1996
Druses of calcium oxalate localized in the anther septum	May play a part in the weakening and eventual enzymatic dissolution of the septa.	Bonner and Dickinson 1989, Horner and Wagner 1980

Table 3 (continued)

Structure/devices and events involved	Structure/device type and manner of involvement	References
Increasing osmotic pressure of epidermis and endothecium cells	The osmotic pressure of the epidermis and endothecium cells increases owing to starch hydrolysis.	Keijzer 1987a
Water translocation from the loculus towards other parts of the anther or the flower	The <i>Lilium</i> anther does not dehydrate and open when the filament is stretched with a thin wire. The hydrolysis of connective cells amyloplasts causes water uptake from the loculus in <i>Cucurbita pepo</i> . Obviously always coupled with heat; is responsible for inducing anther opening; no data on the separate effect of light and heating are available.	Heslop-Harrison et al. 1987 Nepi and Pacini unpublished
Light	High temperatures induce anther evaporation.	Vesprini and Pacini (in press)
Temperature	<i>Helleborus foetidus</i> and <i>H. bocconeii</i> , which blooms during January–March have flowers with around 100 anthers, the higher the temperature the higher the number of anthers exposing pollen and the less the mean life of the flower.	Bianchini and Pacini 1996, Lisci and Pacini 1994, Yates and Sparks 1993
Relative humidity	The lower the relative humidity the faster the process of anther opening; besides in <i>Ricinus communis</i> the lower is the RH, the longer the distance reached by pollen grains which are launched.	

expelled by entomophilous plants, such as *Spartium junceum* and other Fabaceae. In these species the anther is already open when the flower is still closed. Subsequent flower opening is caused by either certain parts of the flower dehydrating or by an insect. The latter becomes dusted with pollen causing the pollen to become entrained in the air.

In gymnosperms, the orbicules are the only structures that remain after degeneration of the tapetal cells (Fig. 2a); they may be on the surface of the loculus (*Macrozamia spiralis*, *Pinus pinaster*, *Ginkgo biloba*) or on the pollen (*Taxus baccata*, *Torreya nucifera*) (Pacini et al. 1999). In angiosperms, the presence of orbicules on pollen has not been described, however, they may coexist with pollenkitt (Pacini and Franchi 1993). In many typically entomophilous families, such as Asteraceae and Lamiaceae, only pollenkitt is present; in typically anemophilous families, such as Urticaceae, Cupuliferae and Poaceae, only orbicules are present. Various functions have been attributed to orbicules. The most feasible is valid only for typically anemophilous species and consists of favouring detachment of pollen from the anther as soon as it opens. Because both the pollen and the orbicules consist of sporopollenin, their surfaces have the same physical properties and electrostatic charge causing the pollen grains to jump from the anther (Heslop-Harrison and Dickinson 1969, Keijzer 1987a).

Anther opening is often an irreversible event, after which the pollen is normally exposed to different kinds of dispersal agents (Table 4). In some cases anthers close during the rainy days. In contrast, opening of the flower is more often a reversible process and the flower may close at the end of anthesis or open in the morning and close in the evening a number of times, such as in *Vicia faba* (Perriaman and Marcellos 1988). Some exception has been reported, i.e. *Lilium philadelphicum*, which exposes its pollen for several days, but the anthers close when it rains, presumably to prevent hydration of the pollen (Edwards and Jordan 1992).

Dispersing units

Types of dispersing units in relation to the number of ovules per ovary

With few exceptions, the spores of mosses and ferns and the pollen of gymnosperms, except for the genus *Ephedra* (Fig. 2b), are dispersed singly. In angiosperms, pollen is dispersed singly or in clumps held together in different ways. Pacini and Franchi (1998) listed 13 types of dispersing units. This difference between angiosperms and gymnosperms may be because despite gymnosperm ovules containing up to ten female gametes, only one embryo develops. In contrast, the angiosperm ovary may contain one, hundreds, or even thousands of ovules, as in orchids, and all of them may be fertilized. It follows that if many pollen grains reach a stigma together, all the ovules may be fertilized. If the ovary contains only one ovule, the surplus of pollen grains arriving simultaneously may increase competition between male gametophytes, favouring selection (Ottaviano and Mulcahy 1989). The increase in the number of pollen grains per dispersing unit is matched either by an increase in the number of ovules per ovary or by gametophyte competition (Pacini and Franchi 1999a). Compound pollen is not always associated with an increase in the number of ovules per ovary; for example, in the genus *Typha* there is only one ovule per ovary, yet pollen is dispersed in tetrads (Fig. 2g). In this species, flowers are monoecious and those of the female inflorescence are packed so closely together that the pollen tubes of a single tetrad may not only penetrate the stigma to which it adheres, but also adjacent stigmas (Nicholls and Cook 1986).

Mechanisms of pollen clumping for multiple dispersing units

The various types of pollen clumping in relation to pollination is summarized in Table 5. Dispersing units composed of several grains of pollen are formed in the following ways (Table 5):

1. By viscous fluids normally derived from degeneration of the tapetum (see also Hesse

Table 4. Analytical key for flower and anther opening, pollen exposure and presentation

-
- A. The flower is cleistogamous and pollen is not exposed. It germinates inside the anther, pollen tubes penetrate the anther walls and reach the adjacent stigma (*Lamium* sp, *Viola* sp.)
- B. The flower opens and pollen is exposed
- B.1. Anther bears a pollen dispersal unit composed by hundreds or hundred of thousands of pollen grains, i.e. the pollinarium (Orchidaceae); an insect collects the pollinarium and this can be deposited on only one stigma (compact pollinium) or spread over different stigmas (soft pollinium)
- B.2. Anthers bear pollen dispersal units as monads, tetrads or polyads
- B.2.1. The anther and/or the flowers are opened by the insect.
- B.2.1.1. The insect determines flower opening, anthers are already open (*Spartium junceum*).
 B.2.1.2. The insects open ripe anthers to collect pollen (some Compositae)
- B.2.2. The flower and the anther open by themselves
- B.2.2.1. Anther dehiscence is explosive and pollen is launched.
- B.2.2.1.1. Pollen is launched because a sudden movement of the filament (*Parietaria* and some other *Urticaceae*).
 B.2.2.1.2. Pollen is launched because of a sudden movement of the anther wall (*Ricinus communis*)
- B.2.2.2. Anther dehiscence is not explosive, all the anthers of a flower open contemporaneously, the flower has a short life; one day or even less, (*Cucurbita pepo* and Compositae); or in contrast only a few anthers open per day, the flower has a longer life: from few days to few weeks, (some Myrtaceae, Ranunculaceae and Proteaceae).
- B.2.2.2.1. Pollen, owing to some mechanisms present in the flower is deposited from the anther to other floral parts where it is collected by pollinators; (secondary pollen presentation, Compositae).
- B.2.2.2.2. Pollen is exposed in the anther (primary pollen presentation). If the anther is big and the pollinator is small the anther is visited more than once.
- B.2.2.2.2.1. Pollen is exposed or leaves the anther gradually
- B.2.2.2.2.1.1. because anthers open gradually (gen. *Cistus*)
 B.2.2.2.2.1.2. because anthers are poricidal (Ericaceae)
- B.2.2.2.2.2. all the pollen grains of the flower is exposed contemporaneously (*Lilium* sp., *Cucurbita pepo*).
-

and Vogel this volume): (a). Pollenkitt, the most common mechanism in dicots and monocots (Fig. 2c); (b). Tryphine, known only in the Cruciferae (Fig. 2d); (c). Elastoviscin, found in some Orchidaceae and Asclepiadaceae (Dannenbaum and Schill 1991) (Fig. 2e). There may also be fluids derived from other parts of the anther (De Frey et al. 1992). Pollenkitt may vary in quantity in different species; when not abundant, exine ornamentation is evident; when abundant, it may hide this ornamentation (Fig. 2d). Elastoviscin is always abundant and is found on pollen that has little or no ornamentation (Fig. 2e).

2. By tangling. (a). The pollen itself may tangle if it is filiform as in marine monocots. (b). The pollen may have viscin threads attached to

the exine in the pore region, as in the genus *Fuchsia* (Fig. 2f), or in other areas (Hesse 1983, 1986). (c). The anther may produce filaments that trap pollen (Halbritter et al. 1997 and references therein). In cases b and c, tangling serves to disperse many grains together and to attach the unit to the bodies of pollinators.

3. By common walls. In this way, four pollen grains derived from the same meiocyte (Fig. 2g) or multiples of a tetrad are brought together. In tetrads, the pollen may be held together by exine bridges (Fig. 2g) or by other mechanisms (Knox and McConchie 1986). When tetrads unite to form more complex dispersing units, they may be few, usually four, forming polyads, as occurs in the genus *Acacia* (Fig. 2h) where each grain is always separated

Table 5. The more common types of pollen dispersal unit are grouped according to their gathering mechanism; these mechanisms can act independently or in combination. For each type the dispersing agents and some examples are also reported. Orchidaceae have the highest number of different types of pollen dispersal units; soft pollinia can be of different types

Sticky fluids		Tangle methods	Common walls
Derived from the tapetum	Derived from the anther		
POLLENKITT E.g. Gymnosperms, Betulaceae, Urticaceae and Poaceae	ANTHER MUCILAGE E.g. <i>Tylosema esculentum</i> <u>zoophily</u>	EXTREMELY ELONGATED INTERMINGLED MONADS E.g. Zoosteraceae <u>hydrophily</u> (submarine pollination)	TETRADS E.g. Typhaceae and Juncaceae <u>anemophily</u>
TRYPHINE E.g. Cruciferae		VISCIN THREADS CONTINUOUS WITH EXINE E.g. <i>Oenothera</i> and <i>Fuchsia</i> sp (Oenotheraceae) <u>zoophily</u>	POLYADS E.g. <i>Acacia</i> (Leguminosae) <u>zoophily</u>
ELASTOVISCIN E.g. <i>Cypripedium calceolus</i> (Orchidaceae)		THREADS DERIVED FROM THE ANTHER E.g. <i>Strelitzia reginae</i> <u>zoophily</u>	SOFT POLLINIA E.g. <i>Loroglossum hircinum</i> (Orchidaceae) <u>zoophily</u>
TETRADS WITH POLLENKITT E.g. Ericaceae and Araceae p.p.		TETRADS GROUPED BY VISCIN THREADS E.g. <i>Rhododendron</i> sp. (Ericaceae p.p.) <u>zoophily</u> ≫ <u>anemophily</u>	COMPACT POLLINIA E.g. Asclepiadaceae p.p., Orchidaceae p.p. <u>zoophily</u>

by exine and intine. When there are many tetrads, as in the case of compound pollinia, the tetrads and the internal pollen grains are only separated by intine, and exine is limited to the outer walls of pollen of the outermost tetrads (Pacini and Franchi 1999b). Only isolated tetrads are dispersed by wind, as in the Typhaceae; in all other cases they are dispersed by animals (Table 6).

Although gymnosperms *sensu latu*. are all anemophilous and their pollen is dispersed in monads (Fig. 2a), the pollen sacs of the genus *Ephedra* (e.g. *E. americana*, *E. aphylla*, *E. campylopoda*) are visited by insects. In this genus, the pollen sacs have pores and filaments and the pollen is coated with pollenkitt that forms clumps (Bino and Meeuse 1981, Bino and Dafni 1983) (Fig. 2b).

The most primitive angiosperms were probably all entomophilous (Friis and Crepet 1988) and their pollen was most likely dispersed as clumps held together with pollenkitt. All the other types of dispersing units evolved later (Pacini and Franchi 1996), with occasional returns to anemophily and hydrophily. In the case of the Betulaceae and Urticaceae, of which all species are anemophilous, pollenkitt is absent; in contrast pollenkitt persists in anemophilous species of prevalently entomophilous families (see Table 6 and Lisci et al. 1996).

Some pollen dispersing units, such as monads, tetrads, and polyads dispersed singly, have a constant number of pollen grains. Others, such as soft and compact pollinia, are composed of a relatively constant number of pollen grains. In cases where pollen is held together by viscous fluids or a tangle method, the number of pollen grains per clump varies according to viscosity, pollen diameter, and temperature. The number of pollen grains per clump may decrease in flight when the pollen is dispersed by wind, especially when the wind is strong (Lisci et al. 1996). In the case of clumps that adhere to the bodies of insects, there are no data but presumably the number of pollen grains fluctuates during visitation to flowers,

thus some remain on the stigma, others stick to the insects' body, and still others are lost (Nepi and Pacini 1993). The greater the number of visits an insect makes to plants of a given species, the greater the number of genotypes of pollen it will carry on its body.

Types of dispersing units, pollination and pistils

The type of dispersing unit is related to pollination syndrome and the number of ovules per ovary (Table 6). Most anemophilous plants have only one ovule per ovary.

Many species have only one type of pollination, but others, such as entomophilous species with well exposed anthers, may be both entomophilous and anemophilous (Dafni and Dukas 1986). Other plants, such as certain Ericaceae, have flowers visited by insects for nectar at the start of anthesis, after which pollen is dispersed by wind when the nectary no longer produces nectar and the pollen has become powdery. It leaves the poricidal anthers more readily in this form (Franchi and Pacini 1996).

Pollen presentation

The flower and anthers of cleistogamous flowers do not open (Lord 1981); in all other cases the anthers open, but there are various possibilities:

a. The anther opens completely and the pollen, not held back by pollenkitt, is released at once, as in gymnosperms and plants with male inflorescences in aments, such as the Betulaceae.

b. The pollen is launched by a movement of the anther (*Ricinus communis*) or filament (*Parietaria judaica*) (Bianchini and Pacini 1996).

c. The pollen is held in the anther by pollenkitt, tryphine, viscin threads, or other mechanisms; this type of presentation is known as primary and is perhaps the most common in angiosperms (Hesse and Vogel this volume).

d. The anthers open slowly, exposing the pollen gradually to insects, as in the genus *Cistus*; this is also primary presentation.

Table 6. The more common types of pollination, pollen dispersal units, structural and ecological features. Anemophilous species have a lower number of pollen grains per pollen dispersal unit in respect to entomophilous species. The higher the number of pollen grains per pollen dispersal unit the higher the number of ovule fertilised in the ovary

	Type of pollen dispersal unit	Pollen exposure and dispersal	Pollen flight	Pollination consequences
Anemophilous pollination	Pollenkitt absent; monads (Graminaceae, Cupuliferae, Urticaceae), tetrads (Typhaceae),	Pollen leaves the anther as soon as anther opens	Pollen grain number per volume unit decreases with distance	Pollen grains, if not intercepted by any surface, may be dispersed even great distances; pollen of different plants falls on a stigma
	Clumps of pollen due to pollenkitt; monads grains (Ranunculaceae pp., Euphorbiaceae pp.) or tetrads (Ericaceae)	Pollenkitt is responsible for pollen adhesion to the anther (primary presentation) until a breeze removes pollen clumps	Clumps with a higher number of pollen grains fall next to the anther, those with a lower number fall further away	Grains of a clump, if not split during flight, fall on the same stigma; big clumps from the neighbouring flowers fall on the stigma
Zoophilous pollination	Monads or tetrads forming clumps by pollenkitt, trypfine, viscin threads, elastoviscin	The pollen persists on the anther (primary presentation) until voluntary or involuntary removal by animals; if anther protrudes from the corolla pollen can be removed by a breeze	Some pollen grains/clumps fall from the anther during animal collection, others during flight, or during a visit to another flower	Pollen grains deposited on a stigma derived from a number of flowers is proportional to the number of previous visits the pollinator has made
Polyads (Acacia sp.)	Anthers have septa, each with one polyad; polyads leave the anther as the anther gradually opens	Some polyads fall from the anther during animal collection, others during flight, or during a visit to another flower	A limited number of polyads are deposited on the stigma; the total pollen grain number is very close to the number of ovules in the ovary	
Massulae united in a soft pollinium (Orchidaceae pp.)	The whole pollinium is collected by the pollinator	Massulae, because loosely attached to the caudicle, can be lost during the flight and visits to other flowers	A limited number of massulae are deposited on the stigma; the number of total pollen grains of the pollinium are very close to the number of ovules in the ovary	
Compact pollinia (Asclepiadaceae pp. and Orchidaceae pp.)	The whole pollinium is collected by the pollinator	Owing to the compact pollinium, pollen loss during the flight is avoided	A very limited number of pollinia are deposited on the stigma; the total number of pollen grains in the pollinium is very close to the number of ovules in the ovary	

e. The pollen is presented to dispersing agents in other parts of the flower (Table 4). This type of presentation is known as secondary (Yeo 1993) and may be due to three different situations. The first is that observed in the Asteraceae and all allied families having flowers organized in inflorescences with small, closely packed flowers (Pacini 1996b). In these situations, there is little space for exposing pollen to dispersing agents, and the anthers are arranged vertically, remaining closed in the corolla tube. For this reason the anthers open inwardly; the pollen adheres to the style by means of pollenkitt; and as the style lengthens it carries the pollen out of the flower (Pacini 1996b). The second situation, which is very similar but has a different outcome, is observed in certain Araceae, such as *Anthurium*. Here the inflorescence is monoecious and the male flowers are closely packed in a cylinder under the spadix. The ripe pollen is extruded from an opening by a piston-like mechanism (Westerkamp 1989). The last mechanism is to enable controlled pollen loading onto the body of pollinator by forcing it to follow a certain path within the flower; hence the pollen must be in a suitable position to attach to the pollinator. This mode is encountered in Araceae such as *Arum* where trapped insects are dusted with pollen falling from the anthers onto the spathe (Faegri and van der Pijl 1979).

For pollen to be transferred from the anther to another part of the flower, transport mechanisms must be present; Westerkamp (1989) identified three such mechanisms that differ in the manner that the pollen is released from the anther and in the mode of pollen presentation. Howell et al. (1993) identified nine classes of secondary pollen presentation, based on the position of the anthers in the flower and on the site of pollen presentation.

Yeo (1993) gives a detailed systematic account of secondary presentation, identifying 19 families of dicots and five of monocots in which this phenomenon occurs. In any case, pollen transport from the anther to other parts of the flower is possible by virtue of pollenkitt,

which initially keeps the pollen in the anther and subsequently at the site of secondary pollen presentation (Pacini 1997).

The pollen of a flower may be presented all at once (Orchidaceae, families with few anthers) or the anthers may be many, some of which open every day (certain Ranunculaceae such as *Ranunculus*, *Anenome*, *Helleborus*). There is also the case in which the anthers open slowly, exposing pollen gradually; the flower may therefore be visited many times by pollinators (Vogel 1983) which then may convey successive waves of pollen to the stigma (Table 4). This occurs in at least two ways: the first is that of poricidal anthers, as in Ericaceae, Violaceae, etc., and in the second the anthers may open gradually as in certain Cistaceae. When these mechanisms exist, pollen forms clumps with pollenkitt and the ovaries contain many ovules.

Pollen on insect bodies

Dispersing units may adhere to pollinator bodies in many ways or combinations of ways. The same mechanisms normally also keep the pollen in clumps; it is rare that they have the exclusive function of adhesion to pollinator bodies. The mechanisms are: (a). Viscous substances produced by tapetal degeneration: pollenkitt, tryphine, elastoviscin (Fig. 2b–e) or anther connective tissue, as in *Tylosema esculentum* (Caesalpiniaceae) (de Frey et al. 1992, see also Hesse and Vogel, this volume). (b). Threads connecting pollen grains, either continuous with exine, as in the case of viscin threads (Fig. 2f) or produced by the anther (Rose and Barthlot 1995). (c). Organization of pollen in pollinia with a sticky appendage, namely the viscidium in Orchidaceae (Fig. 2i) and the corpuscle in Asclepiadaceae. (d). Electrostatic charge that builds up on the pollinator during flight and attracts pollen (see also Vaknin et al. this volume). (e). Sticky parts of the pollinator's body. (f). Hairy or downy parts of the pollinator's body that trap pollen.

Pollen transport by insects has disadvantages, and only part of the pollen remains viable

(Stanley and Linskens 1974). Bees and other hymenopterids, such as ants, have metapleural glands that secrete volatile substances that kill pollen (Beattie et al. 1985, Harris and Beattie 1991, Mesquida and Renard 1989). Pollen collected by bees as food for the larvae is manipulated by addition of the nectar and enzymes, such as invertase that cleaves sucrose and depolymerizes fructans (Stanley and Linskens 1974). This causes a loss of sucrose and fructans from the pollen and an increase in pollen osmotic pressure, which upsets pollen homeostasis. In addition the longer the pollen adheres to the insect, the greater the loss of germination capacity (Stanley and Linskens 1974). Only orchid pollinia survive extended transport by insects, because they are attached by the caudicle, which may be up to 16 mm long (Nilsson 1992), i.e. far from the insect's body.

Conclusions

In gymnosperms pollen grains are dispersed singly and may consist of a variable number of cells depending on the systematic group, but is almost invariably anemophilous (Pacini et al. 1999). In angiosperms, pollen grain consist of two- or three- cells and have different types of pollen dispersing units and vectors. The extreme morphological diversity of angiosperm pollen and its vectors are the result of a number of factors: ovaries may contain many ovules; there are many mechanisms to avoid or reduce competition by attracting pollinators; pollinators are promoted to carry enough pollen to fertilize all the ovules.

Most gymnosperm pollen has a low water content and is dormant at dispersal, whereas some angiosperm pollen has a water content of around 70%, like the cells of most tissues, and is therefore not dormant. This implies that it must find a compatible stigma to emit pollen tubes within a few minutes. This physiological characteristic of pollen increases the possibility of gametophytic competition, which appears to have been determinant in the evolution and success of the angiosperms (Mulcahy and Bergamini-Mulcahy 1987). In contrast, when

the number of ovules per ovary increases, male gametophyte competition decreases and female gametophyte competition increases (Pacini and Franchi 1999a).

The first angiosperms were entomophilous (Frees and Crepet 1988), but in the course of the evolution, there have been many returns to anemophily and even hydrophily. These returns to the past may have been made to avoid competition between species or to attract pollinators and, in temperate zones, to enable pollination in the cold months when insects are scanty or absent. The different types of dispersing units and modes of dispersal have distant polyphyletic origins. For example, the seaweeds are probably derived from a family very close to the Poaceae (Larkum and den Hartog 1989), in which the pollen was only slightly dehydrated. This feature facilitated sub-marine pollination. The Orchidaceae have different types of dispersing units. In those with pollen in monads with pollenkitt, such as *Pterostylis plumosa*, the pollen grains in the tetrads remain close together during development and there is very little space between tetrads (Pandolfi et al. 1993). Further juxtapositions and aggregations have led to the various types of pollinia.

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Cytochemistry of mature angiosperm pollen

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Abstract. The problems involved in applying histochemical and cytochemical methods to mature angiosperm pollen for bright light and fluorescence microscopy are discussed. These methods can be used for general examination or to reveal particular structures or groups of substances. The main methods of testing pollen viability and germinability based on stains and semiquantitative methods are also reviewed. The main methods of staining and their applications are summarised.

Key words: Pollen cytochemistry, pollenkitt, exine, intine, reserves, generative cells, pollen viability.

Introduction

Biochemical methods are often used to determine the chemical composition of cell content. These quantitative techniques usually involve homogenisation of tissues and separation of the various components for analysis. This type of investigation can provide no information about the histological and cytological localisation of the substances detected. To obtain such information, histochemistry and cytochemistry are used. Since these qualitative techniques rarely yield quantitative data, the biochemical and histochemical approaches are complementary.

To make detailed observations, the study material must be fixed, embedded and cut in thin sections. Much has been written on the subject, which is part of histochemistry in the

broad sense. Once the material has been sectioned, it may be treated with different stains, depending on the substances to be visualised. This is histochemistry in the strict sense.

This chapter does not provide an exhaustive account of all cytochemical methods applicable to pollen, but describes those methods – including not strictly cytochemical microscopic techniques, such as polarised light and phase contrast microscopy or pollen germination – which are easily applied in most laboratories concerned with pollination biology. Only classical LM techniques are discussed, and therefore not TEM or immunologic methods. The paper is targeted to peculiar pollen components, and therefore does not deal with the detection of common organelles, for which the usual cytochemistry treatises may be referred to.

The terminology proposed by Punt et al. (1994) for the various structural components of pollen is used.

Pollen and its constituents

Pollen is the male gametophyte of *Spermatophyta*. Angiosperm pollen consists of two or three cells at anther dehiscence, depending on the timing of the second haploid mitosis, which in its turn depends upon the species. For

tricellular pollen, the second haploid mitosis occurs before the anther opens. For bicellular pollen, it may occur: 1) after pollination but before germination; 2) immediately after germination on the stigma surface (the most common case in angiosperms); 3) when the pollen tube reaches the embryo sac. (Stanley and Linskens 1974). The number of cells in mature gymnosperm pollen varies to a large extent (Pacini et al. 1999).

The cells are identified by different shape and size and have walls of different types. The generative cell, in the case of bicellular pollen, or the spermatic cells, in the case of tricellular pollen, are identified by thin pectocellulose walls similar to the common walls of other vegetative cells but in some cases it contains callose (Heslop-Harrison 1968, Tanaka 1988). The vegetative cell, which contains the latter, has a much more complex wall known as the sporoderm (Fig. 1); see also Hesse, in this volume.

The sporoderm consists of two components: the exine (outermost) and the intine. The exine has different parts (Fig. 1), the development of which varies from species to species. In some cases, the exine may be discontinuous or absent (exineless pollen) (Kress 1986). The sporoderm has specialised regions known as apertures where the exine is generally thinner and the intine thicker. The apertures differ in form, number and structure (Thanikaimoni 1986) and are the sites of pollen tube emission and of exchange of water and other substances (Heslop-Harrison

1979a). In addition they play a main role in harmomegathy (Pacini 1990), that is in the changes in volume and shape of pollen grains during dehydration, dispersal and rehydration (Wodehouse 1935). In species with entomophilous, and sometimes in those with anemophilous pollination, the pollen surface is covered by varying quantities of a viscous substance, pollenkitt, which has many functions (Pacini and Franchi 1993, Pacini 1997; see also Kevan et al. and Dobson, this volume).

The cytoplasm of the vegetative cell of mature pollen may contain all the organelles typical of plant cells, except for vacuoles and chloroplasts (but it contains other types of plastids). Generally it is said that vacuoles are absent because the pollen undergoes dehydration in the anther once morphological development is complete and before anther dehiscence. Nevertheless vacuoles are present in pollen of marine monocots and their presence in very few terrestrial plants must be reconsidered (see Pacini and Ackerman, this volume). The cytoplasm may contain reserves of lipids and carbohydrates but not reserves of proteins (Franchi et al. 1996). The existence of stored messenger RNA was demonstrated in mature ungerminated pollen by Frankis and Mascarenas (1980).

Pretreatment of pollen

Ideally, cytochemical studies should be carried out on living, intact material so as to modify

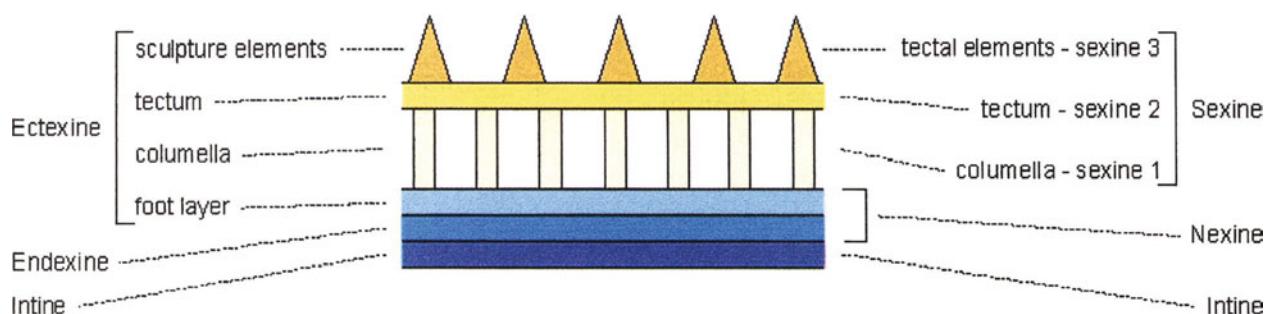


Fig. 1. Schematic representation of the pollen wall layers. The graphic was taken from the electronic version of "Glossary of pollen and spore terminology" (Punt et al. 1994) updated by Peter Hoen. Terminologies are derived from Erdtman (1952) on the right and from Skvarla and Larson (1966) on the left

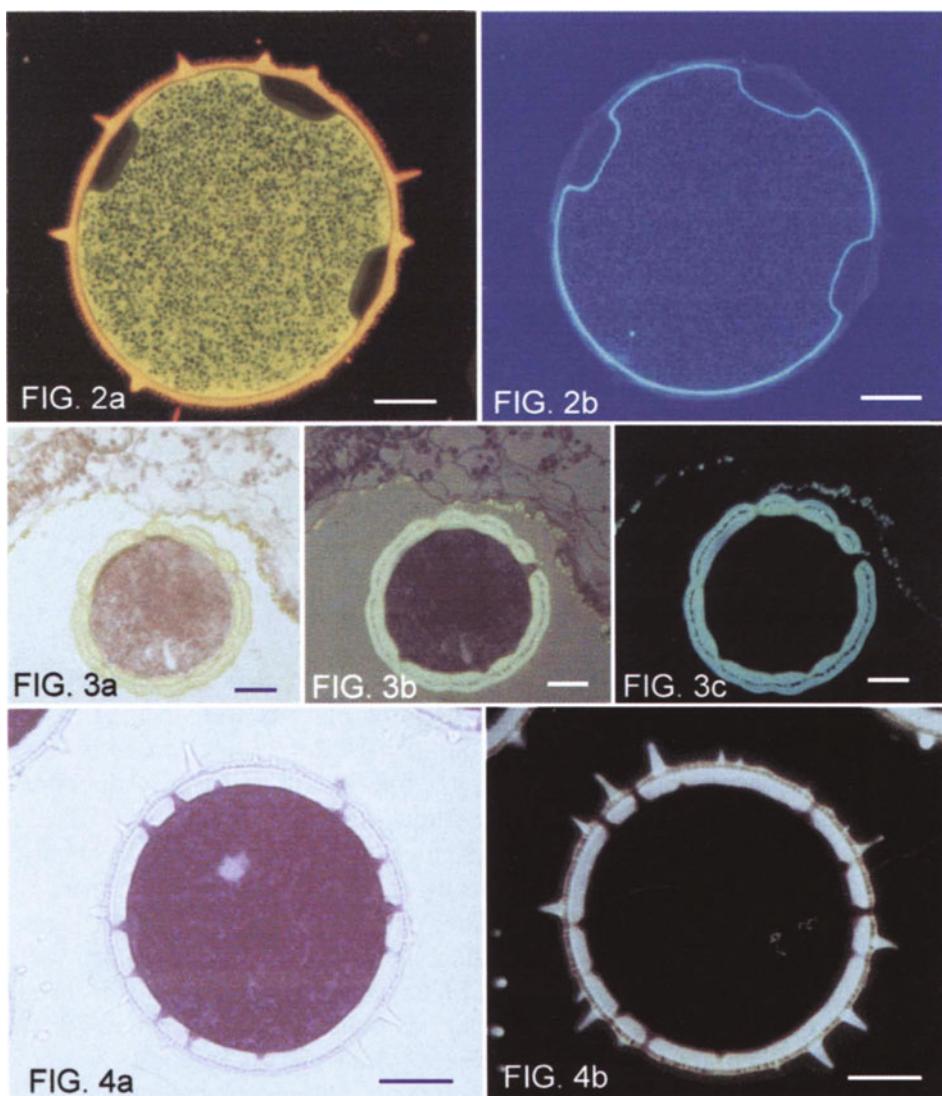


Fig. 2. Section of pollen grain of *Cucurbita pepo* stained with Calcofluor white and basic Fuchsin and observed under: **a** monochromatic blue light; **b** ultraviolet light. The monochromatic blue light evidentiates the exine staining due to basic fuchsin. Some differences are evident in the exine layers, in particular the spine tops and microspines appear brilliant red, while the foot layer has an orange colour. Basic fuchsin acts also as a general cytoplasm stain (light yellow). The ultraviolet light evidentiates the β -glucan layer of the intine. Bar = 20 μ m.

Fig. 3. Section of mature anther of *Mirabilis jalapa* stained by PAS and Auramine O and observed in: **a** bright light; **b** mixed bright and ultraviolet light; **c** ultraviolet light. In bright light carbohydrates reserves are stained by red colour in the cytoplasm, while sporopollenin is only pale yellow. Under ultraviolet light only sporopollenin appears in bright yellow, orbicules are also evident on the loculus surface. The mixed light is useful to observe both stains and to shorten the exposure time. Bar = 20 μ m.

Fig. 4. Section of pollen grain of *Lavatera arborea* stained with PAS and observed in: **a** bright light; **b** ultraviolet light. The PAS reaction evidence the intine and the poral regions as well as starch grains and other polysaccharides reserves in the cytoplasm, exine is unstained. Exine is autofluorescent under ultraviolet light, differences between the foot layer and tectum-columellae are evident. Bar = 20 μ m

the real conditions of the material as little as possible. This is often impossible because single cells can rarely be isolated and reagents have difficulty penetrating the sample. In the case of pollen, the first difficulty does not exist, because pollen consists of only two or three cells and grains are easily separated from the anthers that produced them and from one another, especially in species with anemophilous pollination where pollen is mainly dispersed in monads (Pacini and Franchi 1998); see also Ackerman and Kevan et al., this volume. However, reagent penetration may be impeded by the sporoderm, the thickness of which varies from species to species. The sporoderm may also impede observation of the cytoplasm by virtue of its thickness or affinity for certain stains. Substances outside the sporoderm, such as pollenkitt (see below), may interfere with staining and microscope observation. The pollen protoplast may be isolated using enzymes (Tanaka et al. 1987). In pollen with a sufficiently thin exine, certain stains can be used on fresh material without any pretreatment. It is always difficult to obtain sufficiently detailed observations; it is usually only possible to determine the presence or absence of certain substances. To localise them, it is necessary to fix the material and cut it in 1–5 µm thick sections. Fixation implies alteration of the material and modification of its actual condition.

Many types of fixation have been devised to reduce these alterations. Fixation may be chemical or physical. Chemical fixation uses compounds such as acetic acid, ethanol, formaldehyde, glutaraldehyde and mixtures such as FAA (Jensen 1962). Each fixative has advantages and disadvantages, and the choice depends on what one wants to observe (Jensen 1962). For lengthy storage in a fixative medium the best fixative is FAA; with others, the longer the pollen remains in the fixative, the greater the alterations when using hydrophobic embedding media. Chemical fixation is followed by dehydration, which is classically carried out in an ethanol series with increasing concentration, and by embedding in different

types of resins. Physical fixation involves freezing of the sample which may subsequently be infiltrated with resins or cut directly in the cryostat. Since pollen is small and disperses in the medium, it is often better to collect and treat mature anthers, especially in the case of anthers which are not too large. In this way the anther acts as a container, but there is the risk of collecting anthers which are not completely developed. For chemical fixation and embedding of free pollen, each step in a different medium is followed by centrifuging to precipitate the pollen. For fixation by freezing, the pollen can be previously encased in gelatine (Heslop-Harrison and Heslop-Harrison 1985).

Phase contrast and interference contrast microscopy

Special optical systems make it possible to observe structures *in vivo* and in fixed material, without the use of stains. Phase contrast microscopy exploits differences in thickness and refraction index in the object. These differences cause some rays of light to emerge from the object out of phase with respect to others. The optical system brings out these phase differences, producing an image with contrast. Phase contrast microscopy is often used to observe pollen cultures (Gahan 1984).

Interference contrast microscopy (Nomarski optics) exploits the same principle but light from the source is divided into a beam that passes through the object and a reference beam that by-passes the object, the two beams being recombined prior to entering the eye-piece (Gahan 1984).

Microscopy with polarised light

This type of microscopy can be used to study cell walls and cytoplasmic inclusions such as starch. The waves of a light ray normally vibrate in all planes perpendicular to the direction of propagation of the light. In this technique, a polariser is used to obtain light vibrating in a single plane. Certain substances with molecules orientated in a particular way

cause rotation of the plane of polarised light. Others (known as anisotropic) divide the non polarised ray into two rays vibrating in planes perpendicular to each other. This phenomenon is known as birefringence (O'Brien and McCully 1981). Recently a comparative study of birefringence and staining properties of polysaccharides in pollen amyloplasts has been carried out by Franchi et al. (1996).

Fluorescence microscopy

Many compounds fluoresce when irradiated with UV or monochromatic visible light; they absorb energy and re-emit it at a wavelength displaced towards the red end of the spectrum. Fluorescence may be primary or secondary. Primary or autofluorescence is the natural fluorescence of a component, and is often very weak. Secondary fluorescence is usually more intense and is imparted by fluorescent stains (fluorochromes). Fluorescence microscopes usually have a series of filters to obtain the exact excitation wavelength. Filters also enable fluorochromes with different excitation wavelengths to be used on the same sample (Fig. 2a, b).

Recent fluorescence microscopes have a special illumination system known as epifluorescence. The light irradiates the sample from above and therefore doesn't have to pass through it. Therefore epifluorescence makes it possible to observe thick hand sections of fresh and uncut material. This has great advantages because fixation and inclusion may alter the fluorescent properties of compounds, but are necessary for very small samples such as pollen. It is therefore advisable to choose fixatives and embedding materials that alter the fluorescent properties as little as possible. Fixatives containing heavy metals are preferable to formaldehyde and glutaraldehyde which give the sample a diffuse bluish fluorescence (Gahan 1984). Metacrylate is the best embedding material. Some fluorochromes react with the embedding resin, in this case the resin can be removed to avoid its fluorescence (Gahan 1984, Major et al. 1961). Another common problem is a faint image requiring

long photographic exposure times but some types of fluorescence fade with prolonged irradiation. If the fluorescence is strong, UV and visible light of an appropriate intensity can be used simultaneously. This makes it possible to observe samples treated with both conventional stains and fluorochromes (Fig. 3a, b, c).

Cytochemistry of mature pollen

A lot of stains were developed for or adapted to pollen cytochemistry. A selected choice is presented in Table 1 and discussed underneath.

Pollenkitt

Pollenkitt is an oily substance coating the pollen of many angiosperms, mostly species with entomophilous pollination. It is almost totally absent in gymnosperms (Pacini, this volume). The lipid composition of pollenkitt was studied in 69 species of Californian angiosperms by Dobson (1988), who found that the main differences in composition between species concerned neutral lipids (see also Dobson, this volume). Pollenkitt also contains pigments, mostly carotenoids (Pacini, this volume). Pollenkitt is derived from degeneration of the tapetum, which occurs before anther dehiscence (the exact moment depending upon the species), and is deposited on the surface and in the cavities of the exine (Pacini et al. 1985a, Hesse this volume). Pollenkitt is dissolved by solvents of lipids and must therefore be observed in fresh material. A simple way to check for pollenkitt is to observe pollen in water or aqueous solutions; since pollenkitt is hydrophobic, it forms droplets on the pollen surface in these solution. This method is not suitable for detecting small quantities of pollenkitt. An alternative method is to use SEM to observe untreated pollen and pollen treated with solvents such as chloroform-carbon disulphide (Nepi and Pacini 1993), ether-methanol (Heslop-Harrison and Heslop-Harrison 1982) or cyclohexane; the latter does not alter pollen germinability

Table 1. List of cytochemical techniques for pollen grains, grouped according to specificity. Numbers in superscript in the third column refer to the references cited below

Stain or reagent	Specificity	Stained pollen components	Optics	Technical references
Polysaccharides				
PAS	Insoluble polysaccharides with two adjacent, free hydroxyl groups*	Intine, starch, cytoplasm, sperm cells wall ^{1,2,3,4,5,6,7}	Bright field	O'Brien and McCully 1981
IKI (iodine-potassium iodide or Lugol)	Starch	Amyloplasts ⁵	Bright field	Johansen 1940
Alcian blue 8GX	acid polysaccharides, pectic acid ⁺	Intine ^{2,4,6,8}	Bright field	Jensen 1962
Ruthenium red	acid polysaccharides, pectic acid ⁺	Intine ^{1,2,5}	Bright field	Johansen 1940
Hydroxylamine-iron after methylation	Esterified pectins	Intine ¹	Bright field	Reeve 1959
Zinc chloriodide	Cellulose and hemicellulose	Intine, exine ¹	Bright field	Rawlins and Takashi 1952
Tinopal	Cellulose	Intine ⁹	UV	Regan et al. 1990
Calcofluor White	β -D-glucans (cellulose and callose)	Intine ^{1,2,3,4,8}	UV	O'Brien and McCully 1981
Aniline blue	1,3 β -D-glucans (callose)	Intine, cytoplasm vesicles, pollen tubes ^{1, 2, 3, 4, 6, 8, 9}	Bright field, UV	Johansen 1940, Currier and Strugger 1956
Sirofluor	1,3 β -D-glucans (callose)	Pollen tubes ¹³	Blue-violet, UV	Evans and Hoyne 1982
Lipids				
Auramine O	Lipids (including waxes)	Exine ^{1,2,3,4,6,7,8}	UV	Heslop-Harrison 1977
Scarlet R	Lipids	Exine, pollenkitt, cytoplasm ^{2, 4}	Bright field	Gurr 1965
Sudan dyes	Lipids	Exine, cytoplasm ^{1,2,3}	Bright field	Baker 1947
Nile blue	Lipids	Exine ¹	Bright field	Cain 1947
Osmium tetroxide	Unsaturated fats	Exine, cytoplasm ^{1,2}	Bright field	Cain 1947
Fluorol yellow 088	Lipids	Cytoplasm ⁹	Blue	Regan and Moffat 1990
Proteins				
Aniline blue black	Proteins	Intine, exine ^{1,3,9}	Bright field	Fisher 1968
Coomassie Blue	Proteins	Intine, exine, cytoplasm ^{1,2,4,8}	Bright field	Fisher 1968, Knox 1979

1-anilino-8naphthalene sulphonic acid (1-ANS)	Proteins	Intine, cytoplasm ^{2,3,4}	UV	Heslop-Harrison et al. 1974
Bromophenol Blue (BBF)	Proteins	Intine, cytoplasm ^{2,6}	Bright field	Mazia et al. 1953
Naphthol yellow S	Proteins	Intine, cytoplasm ²	Bright field	Deitch 1955
FITC	Proteins	Intine, cytoplasm ⁷	UV	Pearse 1985
Chloramine T – Schiff	Proteins*	Intine, cytoplasm	Bright field	Jensen 1962
Nucleic acids				
4',6-diamidino-2-phenylindole (DAPI)	DNA	Nuclei ^{4,9,10,11,12}	UV	Goff and Coleman 1984
Mithramycin	DNA	Nuclei ¹⁰	UV	Goff and Coleman 1984
Ethidium bromide	DNA	Nuclei ^{11,13}	Blue-violet	Le Pecq and Paoletti 1967
Hoechst 33258	DNA	Nuclei ¹³	UV	Laloue et al. 1980
General stainings				
Azur B	Negatively charged groups, metachromatic	Vegetative nucleus (DNA), nucleolus (RNA), cytoplasm ^{2, 9}	Bright field	Flax and Himes 1952
TBO	Negatively charged groups, metachromatic**	All components except lipids and starch ^{2,6,7,9}	Bright field	O'Brien et al. 1964
Lacto-phenol Cotton Blue	High density cytoplasm	Cytoplasm	Bright field	Darlington and La Cour 1960
Basic Fuchsin	Negatively charged groups	Exine, cytoplasm ^{1,4,8}	Bright field, UV	Fægri and Iversen 1964

* Staining must be preceded by aldehyde blockade if the tissue has been fixed in aldehydes (O'Brien and McCully 1981)

+ Pectic compounds are localised when present in high concentration. Failure to react may not necessarily indicate an absence of these substances.

Stain for at least 2–3 hours
** When a dye reacts with a tissue element to produce a colour that is different from that of the dye, it is known as methachromatic stain. In the case of Toluidine Blue O the colour of the stained elements depends on their chemical nature: RNA purple; DNA blue or blue-green; polyphosphates, polysulphates, and polycarboxylic acids including alginic acid and pectic acid, red or reddish purple; polyphenols and lignin green or blue-green (O'Brien and McCully 1981)

¹ (Southworth 1973); ² (Heslop-Harrison and Heslop-Harrison 1980); ³ (Ashford and Knox 1980); ⁴ (Heslop-Harrison and Heslop-Harrison 1985); ⁵ (Franchi et al. 1996); ⁶ (Nepi et al. 1995); ⁷ (Nepi and Pacini 1999); ⁸ (Heslop-Harrison et al. 1986); ⁹ (Regan and Moffat 1990); ¹⁰ (Coleman and Goff 1985); ¹¹ (Matthys-Rochon et al. 1987); ¹² (Vergne et al. 1987); ¹³ (Hough et al. 1985)

(Heslop-Harrison and Heslop-Harrison 1985). To detect pollenkitt, fresh pollen can be stained with Scarlet R, pollenkitt appears as red droplets scattered over the surface of the exine (Heslop-Harrison and Heslop-Harrison 1985).

Many functions have been attributed to pollenkitt, some related to efficiency of dispersal by animals and others to survival of the male gametophyte (Pacini 1997). One is the function of forming clumps of pollen grains. Other substances (elastoviscin, tryphine), also derived from tapetum and lipidic in character, fulfil this function too (Pacini and Franchi 1998, Hesse and Vogel, this volume).

Exine

The exine is the most external part of the sporoderm. Its morphology varies widely from species to species but it always consists of sporopollenin, a polymer extremely resistant to physical and chemical agents. Its chemical and biochemical nature is far from completely elucidated (Meuter-Gerhards et al. 1995; Southworth 1973, 1974, 1990; Wiermann and Gubatz 1992). Sporopollenin may be isolated by acetolysis, a technique widely used in palynology (Wiermann and Gubatz 1992), since it is the only component resistant to this reaction (Erdtman 1960). According to this technique polliniferous material is crushed in acetic acid, treated with a mixture of concentrated sulphuric acid and acetic anhydride (9:1) for 5–10 min at 100 °C and then washed with water (Nilsson and Praglowski 1992). Sporopollenin is autofluorescent (Fig. 4a, b) and has affinity for basic stains. Some of the most sensitive of these are Auramine O (Fig. 3a, b, c), a fluorochrome initially used to stain the stigma cuticle (Heslop-Harrison 1977), and basic fuchsin (Fig. 2a). Both stains intensify differences between the parts of the exine (ectexine, endexine). Basic fuchsin is a simple way to identify pollen grains on the stigma or on the bodies of insects (Beattie 1971). It is also used as a fluorochrome which colours the exine bright fluorescent red.

The exine, especially if sculptured, functions as a deposit for materials of sporophytic origin (i.e. derived from the tapetum), such as pollenkitt and proteic substances that play a role in the pollen-stigma interaction which underlies compatibility-incompatibility (Heslop-Harrison 1975, Heslop-Harrison et al. 1975). Sporophytic proteins may be dispersed over the whole surface of the exine or clustered near the apertures (Pacini et al. 1981). There may also be small quantities of polysaccharide residues of primexine (Southworth 1973). Because of the presence of these substances and pollenkitt, the sporopollenin is often contaminated with compounds of a different chemical nature and its structure may also be altered by the drastic treatments necessary to isolate it. This is why it is so difficult to make a chemical characterisation of the exine (Wiermann and Gubatz 1992) and why exine may react to stains for proteins, lipids and polysaccharides (Southworth 1973).

The exine is generally thinner or absent near the apertures (Thanikaimoni 1986). Around the pores, the exine may form an operculum that has different characteristics from the exine located elsewhere (Thanikaimoni 1986).

In angiosperms, beside exine, other structures containing sporopollenin are present, for example Ubisch bodies (or orbicules) and viscin threads (see Hesse and Vogel, this volume). The former usually adhere to the inner tangential walls of tapetum cells and their function is uncertain, however it has been established that they are not temporary stores of sporopollenin (Pacini et al. 1985a). At least in some gymnosperms the Ubisch bodies were found on the surface of pollen grain (Pacini et al. 1999). Viscin threads are filaments of sporopollenin that connect with the surface of several pollen grains and have the function of forming clumps of pollen (Hesse, this volume). They have been found in species of the Onagraceae and Ericaceae and in a few Caesalpiniaceae (Pacini et al. 1985a, Wiermann and Gubatz 1992, Hesse, this volume).

Some Araceae have peculiar structures external to the exine and resembling sculpturing; they are formed by a polysaccharidic sponge containing sporophytic proteins (Pacini 1997).

Intine

The intine forms the internal part of the sporoderm. It is usually thin in between the apertures and thick in the apertures where it forms structures such as the oncus of the hazelnut (Heslop-Harrison and Heslop-Harrison 1991), the Zwischenkörper of the Gramineae (Heslop-Harrison 1979b) and similar structures in some Cucurbitaceae (Nepi et al. 1995). From the chemical point of view interapertural intine is similar to the primary wall of plant cells, since it consists of cellulose, pectins and hemicellulose (Keijzer 1987). The intine usually has a layered structure which is particularly evident near the apertures. The deepest layer usually consists of cellulose and the more superficial ones of pectins (Southworth 1973, Heslop-Harrison and Heslop-Harrison 1991, Nepi et al. 1995). The microfibrillar structure of the intine, however, seems to be heterogeneous, since certain species not only contain the cellulosic β -1,4-glucan but also β -1,3-glucan like callose (Heslop-Harrison 1979a, Heslop-Harrison and Heslop-Harrison 1982, Nepi et al. 1995). To study the morphology of the microfibrillar component of the intine, Heslop-Harrison and Heslop-Harrison (1982) removed the exine by heat treatment in 2-ethanolamine and stained it with Calcofluor white. This stain is specific for β -glucans (Heslop-Harrison and Heslop-Harrison 1985) and gives sharp images of the deepest layer of intine of thin sections of embedded pollen.

Like the exine, the intine contains proteins, but they differ in function and origin (Heslop-Harrison 1979a). They are named gametophytic because produced by the gametophyte itself and are stored mainly in the thick region near apertures, generally in membrane-covered tube-like structures that are sealed at the end

of intine development (Franchi and Pacini 1980, Pacini and Juniper 1979).

Because the cellulose component of the intine is fibrillar in character, it can be observed by phase contrast microscopy and with polarised light.

Reserves in the vegetative cell

Mature pollen contains carbohydrate and lipid reserves. Lipids, if present, are always in spherosomes (Pacini, this volume). Sugar reserves may be of three main types (Franchi et al. 1996, Speranza et al. 1997): 1) soluble carbohydrates dissolved in the cytoplasm (mono-, di- and oligo-saccharides); 2) insoluble carbohydrates (excluding starch) in cytoplasmic vesicles; 3) starch in amyloplasts. Pollen usually contains more than one type of carbohydrate reserves, which vary from species to species and in relation to environmental parameters such as temperature and relative humidity (Stanley and Linskens 1974, Franchi et al. 1996, Speranza et al. 1997). An inverse correlation seems to exist between starch and sucrose content (Speranza et al. 1997). Some soluble carbohydrates, such as glucose, fructose, sucrose and maltose, can be detected histochemically (Johansen 1940, Gabe 1976) but these techniques are not commonly used. All insoluble polysaccharides with two adjacent, free hydroxyl groups can be detected by PAS, and there are specific stains for some of them. Starch stains specifically with IKI (Johansen 1940). Starch consists of two sub-units, amylose and amylopectin, the relative proportions of which affect the physicochemical properties of the molecule and make the IKI stains range from brown to dark blue or black. The birefringence of starch, which is due to the crystalline properties of amylose, is not a constant characteristic (Franchi et al. 1996). Although cytoplasmic vesicles have long been known to contain other insoluble polysaccharides, little is known about their nature and their content (Franchi et al. 1996). In pollen of many species, polysaccharides of the fructan family were recently detected (our unpublished

data) by a histochemical technique for inulin (Johansen 1940), which is the most well-known molecule of the family. Fructans are compounds derived from polymerisation of fructose on a sucrose primer; their length ranges from three to hundreds of monosaccharide units (Kandler and Hopf 1980). They are known as reserve substances of tubers and rhizomes and also to a much lesser extent of seeds (Kandler and Hopf 1980). Beside acting as reserves, fructans act as osmoregulators via their polymerisation and depolymerisation (Edelman and Jefford 1968). They play an important role in low-temperature tolerance, water stress and regulation of water uptake (Kandler and Hopf 1980, Hendry 1993, Spollen and Nelson 1994).

The type of reserve substance is related to pollen size and the type of insect pollinator (Baker and Baker 1979). Small pollen generally contains lipids rather than starch. Pollen collected by dipterans and hymenopterans is generally without starch (Endress 1994:p. 150) as well as pollen collected by bees (Franchi et al. 1996).

Generative cell and male gametes

Two different mitoses occur in the microspore, which in its turn originates from meiotic division of the microspore mother cell. The first haploid mitosis is an unequal division that gives rise to the larger vegetative cell and the smaller generative cell. The latter subsequently undergoes a second haploid mitosis to produce two sperm cells of similar morphology. As mentioned in the introduction, the second haploid division may occur at different times and mature pollen may be bicellular (vegetative cell and generative cell) or tricellular (vegetative cell and two sperm cells). In many species, a physical association has been observed between the sperm cells and the vegetative nucleus, giving rise to the male germ unit (MGU) (Russel et al. 1990). It was initially difficult to characterise the sperm cells because they are situated in other structures (the pollen grain or pollen tube). Russel (1986) was the

first to isolate sperm cells of *Plumbago zeylanica* and from that, different isolation techniques have been developed (Tanaka 1993). The sperm cells have a condensed nucleus and a reduced quantity of cytoplasm. They are surrounded by a sinuous cell wall of uniform thickness between two cell membranes: one of the vegetative cell and the other of the gamete cell (Tanaka 1993). The wall of the generative and sperm cells consists of polysaccharides and generally is stained by PAS. Tanaka (1988) detected callose in the generative cell wall of *Lilium longiflorum* by staining with aniline blue. Using the DNA-specific fluorochrome DAPI (4,6-diamino-2-phenylindole) to study male cytoplasmic inheritance, Miyamura et al. (1987) showed that, at least in certain species, mitochondria and plastids were excluded from male gamete cells during maturation, but this observation is not in line with the detection of organelles containing DNA by hybridisation with specific probes (Corriveau and Coleman 1991).

DNA-specific fluorochromes such as DAPI and mithramycin are useful for assessing the nuclear state in microspore and pollen development and germinating pollen tubes, since they are much faster than Feulgen staining and more specific than aceto-orcein and aceto-carmine (Coleman and Goff 1985). They have the advantage of being vital stains, but they also function with fixed material. To facilitate penetration of the pollen grain or tube, a permeabilising agent such as Triton X-100 can be used (Vergne et al. 1987). These two fluorescent stains have also been used to quantify DNA in fixed pollen by microspectrofluimetry (Coleman and Goff 1985).

Semiquantitative stains

Because of their small size, whole pollen grains can be used in various types of test and can be observed under the microscope. The only exception is very large pollen and pollen with very thick exine and intine. All the problems associated with embedding and cutting of other materials can therefore be avoided and

various evaluations valid for single pollen grains can be carried out.

From the cytochemical point of view, specific stains always refer to groups of substances with similar chemical properties, the molecules of which can, however, differ in a large extent (substitutions, degree of polymerisation, and so forth). This is why there are no truly quantitative stains for which the intensity of colour enables quantification of stained substance by spectrophotometric analysis, or the like. However there are stains based on precise chemical reactions which are perfectly reproducible as far as colour intensity is concerned and can therefore be used statistically in a semiquantitative manner. In other words, a semiquantitative reaction can be used to calculate the percentage variation in colour intensity, and thus of the content of a certain group of substances (big groups like total proteins or total polysaccharides), with respect to a reference sample (Pearse 1985).

Some stains that can be used in this way are based on the Schiff reaction. Artefacts are avoided by blocking free aldehyde groups (O'Brien and McCully 1981). Stains suitable for pollen include the periodic acid – Schiff reaction (PAS) for total polysaccharides and chloramine T – Schiff reaction for total proteins (Jensen 1962); to evaluate the intensity of staining, measured in arbitrary units, the microspectrophotometer (Franchi et al. 1997) and cytodensitometer (Pacini and Viegi 1995, Pardi et al. 1996) have been used. Readings are made at 560–565 nm. It should also be possible to use other stains based on the Schiff reaction for similar purposes.

In theory, other types of stain may be used semiquantitatively. Since most stains are not based on a stoichiometric reaction (that is a reaction with a fixed numerical relationship between the reactants and the products) but on the adsorption of a chromophore, to have a good reproducibility the greatest attention must be paid to the quantity of stain that comes into contact with the pollen and to the uniformity of contact, possibly staining the various samples altogether. Staining for starch

with IKI solution (Johansen 1940) has been used semiquantitatively in pollen (Speranza et al. 1997).

Certain fluorochromes lend themselves to semiquantitative applications. Coleman and Goff (1985), for example, determined the quantity of DNA in pollen by microspectrofluorimetry after treatment with mithramycin or DAPI.

It is worth recalling that the small size of pollen allows an easy extraction of many contained molecules or an easy penetration of reagents. Therefore pollen can also be studied by biochemical methods (Hoekstra and Van Roekel 1988, Speranza et al. 1997), an easier approach to solve many quantitative analytical problems.

Pollen viability and germinability

The need to quantify the capacity of pollen to fertilise arose with the cultivation of plants, especially those grown for their fruits or seeds, and with the development of plant breeding in order to check the quality of temporary stored pollen. Failure to fertilise the ovules in the ovary and the resulting (with few exceptions) failure of the ovary to become a fruit, may depend on many factors. The female part may be sterile, for various reasons, or there may be incompatibility (interspecies, intergenus and self-incompatibility) with the pollen being recognised as foreign (or "self", in the case of self-incompatibility) by the female part, and its germination or development blocked at different levels (De Nettancourt 1977, Williams et al. 1994).

In other cases, fertilisation failure may be due to the pollen (male sterility). This may be total or partial, and due to various factors: genetic and hence irreversible (hybridism, polyploidy, mutations as a result of excessively protracted vegetative reproduction), environmental (e.g. the effects of rain, temperature and relative humidity on pollen dehydration), and experimental (irradiation, treatment with chemical products) (Laser and Lersten 1972, Halterlein et al. 1980, Eeninck 1981,

Franchi et al. 1984, Pacini and Franchi 1987, Bassani et al. 1994, Lisci et al. 1994).

It is therefore extremely important, from the point of view of agronomy, as well as pollination biology, to be able to check pollen fertilisation capacity. Various cytochemical methods, which differ in conception and reliability, have been developed for this purpose (see Dafni and Firmage, this volume).

The simplest ones are based on general cytoplasmic stains, such as lactophenol Cotton Blue (Darlington and La Cour 1960) and the stain of Alexander (1969), and the concept that pollen devoid of cytoplasm is dead pollen. Dead pollen grains do not stain or stain differently from those with cytoplasm. These methods are easy to use but do not always give reliable results. Indeed, "pollen grains which remain unstained lack a protoplast and are definitely aborted" (Jones 1976), but "those with cytoplasm are not necessarily fully fertile" (Ockendon and Gates 1976), which means that these methods overestimate pollen viability (Mulcahy 1981). Pollen viability depends on the timing and manner of pollen degeneration (for example, pollen anomalies in cultivated plants; see Pacini et al. 1978, 1985b and Chichiricò and Grilli Caiola 1982). If the data obtained using morphological methods is to be reliable, a single count is not enough, but the question must be investigated in greater depth, for example as proposed by Franchi et al. (1984), who decided to consider unviable not only grains lacking cytoplasm, but also those: a) with scanty cytoplasm not filling the whole grain; b) with a degenerating cytoplasm, i.e. a non homogeneous intensity of cytoplasmic staining, not due to the generative cell or the organelles; c) with a plasmamembrane detached from the intine, even in a restricted area (early sign of plasmolysis). The method is longer but should be as reliable as enzyme methods, which are widely regarded as the best (Franchi et al. 1983, 1997; Bellani et al. 1985a, b; see also Dafni and Firmage, this volume).

Another method claimed as quite accurate is based on staining proline with isatin (Pálfi and Mihalik 1985): the intensity of colour

should provide an indication of pollen fertility. The method cannot be applied to species, like *Helianthus annuus*, that have a low proline content. For further comments on this method and its reliability, see Dafni and Firmage, this volume.

Enzyme methods are based on colour reactions induced by enzyme activities in the pollen grain. They are based on the assumption that if the activity is present, the colour reaction occurs and the pollen is viable. The first methods used for this purpose were based on tetrazolium salts that change colour when reduced by respiratory enzymes in the mitochondria (Hauser and Morrison 1964, Stanley and Linskens 1974). Initially developed to check the viability of other types of cells, they can also be applied to pollen.

The most widely used of these methods is that proposed by Heslop-Harrison and Heslop-Harrison (1970) and modified by Shivanan and Heslop-Harrison (1981). This technique is known as FCR (fluoro-chromatic reaction), it is based on entry of the nonpolar substrate fluorescein diacetate into the vegetative cell where it is hydrolyzed by esterase to a polar product, fluorescein, which is retained by the cell membrane. When examined by fluorescence microscopy, pollen with an integral plasmamembrane is fluorescent and is counted as viable; non fluorescent pollen grains are considered not viable. The method is fast and lends itself to routine use on large quantities of pollen, however it overestimates germination capacity "because certain cytological events (for instance, failure of the first haploid mitosis) and degeneration are unrelated to the activity of this particular enzyme" (Franchi et al. 1984). Limited correlations between pollen stainability with enzymatically induced dyes and pollen germinability are also reported by Werner and Chang (1981) and Barrow (1983). Since the plasmamembrane of strongly dehydrated pollen is partially disorganized (Heslop-Harrison 1979c), it is sometimes necessary to perform a preliminary rehydration (see Dafni and Firmage, this volume, Table 2).

More realistic values can be obtained by germinating pollen under appropriate conditions and calculating the percentage of germinated grains. When it germinates, pollen emits a pollen tube, the length of which is measured in "diameters", namely pollen diameter. A pollen grain is regarded as having germinated when pollen tube length exceeds one diameter. The pollen is germinated *in vitro* at controlled temperature, using a culture medium containing salts (for which there are many recipes, the most widely used being that of Brewbaker and Kwack [1964]) and 5–15% of sugars. The optimum percentage of sugars should be determined for each species, otherwise 10% is used. Furthermore, sugar percentage may more or less affect both germination time and the presence of starch in germinating pollen (Bellani et al. 1985a, b). Grains which germinate are visible under the microscope without any special techniques. General cytoplasmic stains, phase or differential interference contrast can be used if necessary.

Germination *in vitro* requires the time necessary for the pollen to rehydrate, but is faster than *in vivo* (e.g. 45' versus 3 h 30' in *Lycopersicum peruvianum* Mill. (Cresti et al. 1977), and much faster for pollen which is not, or only slightly, dehydrated (Nepi and Pacini 1999).

The fastest way of producing pollen tubes *in vitro* is the "instant pollen tubes" method (Linskens and Mulleneers 1967). However, rather than true pollen tubes, they are cytoplasmic protrusions in structures similar to pollen tubes caused by osmotic or pH shock. This method is more rightly considered a morphological method because it only checks for cytoplasm, not cytoplasmic function, and is, thus, rarely used.

Finally, germinability of pollen may also be measured *in vivo*. The pollen tube rapidly penetrates the stigma and style. The tissues of the stigma and style must therefore be digested to reveal the tubes. There are a few exceptions to this rule, for example feathery stigmata so small in section that the tube runs externally. After digestion, the tubes can be detected by

fluorescence of callose using aniline blue (Martin 1959), or may be stained with lactophenol Cotton Blue, though internal vacuoles may impede visualisation of the pollen tube path. The pollen tubes can also be detected by the method of Alexander (1987).

In vivo methods give the most realistic results, but forms of competition exist *in vivo* between pollen at various levels. Germination is also affected by mass effects, and irrespective of the number of ovules per ovary, a "minimum pollen load" is often necessary to ensure fertilisation (Lisci et al. 1994, Pacini and Franchi 1999). This is why even the most accurate tests and exclusion of male sterility are never one hundred percent reliable for predicting fertilisation (fruit set and seed set).

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The ecology and evolution of pollen odors

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Abstract. The literature is reviewed and new evidence presented that pollen produces odors, which serve multiple functions in pollination and defense. Pollen odor, which originates from pollenkitt, comprises volatiles that belong to the same chemical classes found in flower scents, that are in species-specific mixtures, and that contrast with odors of other floral parts. Pollen can also take up volatiles from surrounding floral odors, but this adsorption is selective and varies among species. Pollen odors are more pronounced in insect- than bird- or wind-pollinated plants, suggesting that volatile emission evolved in part under selection to attract pollinators. Pollen-feeding insects can perceive pollen odor and use it to discriminate between different pollen types and host plants. Pollen odor influences bee foraging, including the location of pollen sources, discrimination of flowers with different amounts of pollen, and host-plant recognition by pollen-specialist species. In the few wind-pollinated plants studied, odors of male flowers or pollen are comparatively high in α -methyl alcohols and ketones; these volatiles may serve in pollen defense, with some known to repel insects. Pollen odor often includes chemicals with documented defense activity, which is probably aimed mainly at nonpollinator pollen-feeding insects and pathogens; an involvement in pollen allelopathy is also possible. Pollen volatiles comprise chemically diverse compounds that may play multiple roles, and their emission in pollen odor undoubtedly evolved under the principle, and often conflicting, selective pressures to both

protect the male gametophyte and increase its dispersal by animals.

Key words: Pollenkitt, volatiles, flowers, plant defense, pollen foraging, pollination.

Following the heightened interest in pollination spurred by the publication in 1793 of Sprengel's "Das entdeckte Geheimnis der Natur im Bau und in der Befruchtung der Blumen", pollen received increased recognition for its role in pollinator attraction and plant reproduction (see Wodehouse 1935), but no mention of pollen odor appeared in the literature until the 1920's. Odors arising from anthers or pollen are often considered to represent the oldest food attractants for flower visitors (Crepel 1983) and to have preceded other olfactory and visual cues (Porsch 1954, Faegri and van der Pijl 1979), although floral secretions may have played the central attractive role in flowers that offered rewards other than pollen (Endress 1994a). Pollen odors most likely evolved as a defense against pathogens and pollen-feeding animals, prior to the development of animal pollination, following an evolutionary scheme suggested by Pellmyr and Thien (1986). As flowering plants became dependent on animals for pollen transfer, there would have been increasing selection pressure for pollen odor to include

volatiles attractive to the pollinators, which may have included both food-related and mating-related chemical cues.

Pollen of many species has since been described as having distinctive aromas as evaluated by the human nose (von Frisch 1923; Porsch 1954; 1956; von Aufsess 1960; Buchmann et al. 1977; Faegri and van der Pijl 1979; Buchmann 1983). In some plants, including mainly beetle-pollinated taxa in the Magnoliaceae (Thien et al. 1975) and Ranunculaceae (Pellmyr et al. 1984) and bee-pollinated taxa in the Solanaceae (D'Arcy et al. 1990), floral aroma is produced exclusively by the androecium, but the contribution of pollen in these examples still remains to be established. Meanwhile, over the last 10 years chemical analyses of volatiles in pollen headspace (i.e. air surrounding the pollen) in a dozen species have confirmed that angiosperm pollen has species-specific odors and that these are chemically distinct from the odors emitted from other floral parts (Dobson et al. 1990, 1996; Bergström et al. 1995). The chemical confirmation that pollen emits odors has given new impetus to studies of the role of pollen odor in pollination ecology, especially in attracting flower-visiting insects and modulating their foraging behavior, and in defending pollen against pollen-feeding organisms and pathogens (Dobson 1994, Dobson et al. 1999).

Perhaps most important in our attempts to shed light on the evolutionary significance of pollen odor is the need to keep in mind the two simultaneous conflicting pressures faced by the plant with regard to its pollen, namely the need to both protect it from over-exploitation by non-pollinating insects and pathogens and to advertise it as an attractive reward to pollinators. It is also possible that some of the volatiles may play a role in pollen-pistil recognition or interfere with such interactions through allelopathic effects (see section on defense). Pollen odor undoubtedly serves multiple functions, with the emission of individual components having evolved under different selection pressures. The advantage to plants in having their own particular pollen odor varies

with each plant's reproductive biology and needs to be evaluated on a species-by-species basis.

Pollenkitt as the source of pollen odors

Knoll (1930) was the first to address the origin of pollen scents and insightfully proposed that pollen odor arises from the pollenkitt. Knoll coined this term for substances that cause pollen grains to clump, more specifically in reference to the oily and often sticky, colored coating on the outside of pollen grains. Up to then, pollenkitt had attracted only spotty attention. Observations that pollen grains are enveloped in an oily coating were made by early students of pollen biology (Kölreuter 1761, Fritzsche 1837, von Mohl 1852). In the later 1880's, Fischer (in Wodehouse 1935) and especially Kerner von Marilaun (1898) recognized the role of pollenkitt in increasing pollen adhesion during pollination by animals, a function emphasized with the renewed interest in pollination biology in the 1920's (Parker 1926, Troll 1928). Knoll (1930) devoted an extensive discussion to the various functions of pollenkitt, the most notable being that it enhances pollen adhesion, provides yellow color that visually attracts flower visitors, serves as a valuable nutritious substance to pollen-feeding insects through the fatty oils it contains, and, most important here, that it contains the pollen odor. Indeed, Knoll appears to be the first to suggest that substances constituting the basis of pollen odor, which he points out to be of utmost importance to insects in locating and collecting pollen, reside in the pollenkitt. In the 1950's, pollenkitt oils were proposed to be located externally to the pollen protoplasm (Steffen 1953), which was confirmed with the development of electron microscopy (Pankow 1958). In subsequent extensive studies, Hesse (1980, 1981) established that while pollenkitt appears to be universally present in angiosperms, it varies in its distribution on the pollen surface, thereby conferring varying degrees of stickiness in association with different pollination

strategies. Pollenkitt is currently considered to play a multitude of functions besides providing pollen odor and olfactory attractants to pollinators (Dobson 1989, Pacini and Franchi 1993), including: protecting the male gametophyte against ultraviolet light, desiccation, and pathogens; aiding in pollen clumping and adhesion to animal vectors; providing visual attractants, as well as nutrition and phagostimulants to pollinating animals; and participating in pollen-pistil interactions following pollination (e.g. pollen adhesion to stigma, pollen hydration, germination, and pollen-tube growth).

Pollenkitt is thought to contain most of the lipid diversity in pollen, and thus to comprise the majority of non-polar ether-extractable materials, which represent 1–20% (but usually ≤5%) of the pollen dry weight (Barbier 1970, see Stanley and Linskens 1974). In anemophilous taxa (e.g. Poaceae), the values typically fall below 2% (Wittgenstein and Sawicki 1970, Bianchi et al. 1990). However, unless the extraction is very rapid (less than 30 seconds) to exclude the removal of internal lipids (Knox and Heslop-Harrison 1970, Heslop-Harrison et al. 1973), ether extracts will contain lipid material from the entire pollen grain, including the inner wall (intine), cell membrane, and protoplasm, rather than from only the exine surfaces and cavities where the pollenkitt resides. In 1989 we conducted a study to obtain more accurate estimates of the contribution of pollenkitt to pollen weight. We washed pollenkitt from the pollen of four species using the method in Dobson (1988), where hand-collected pollen in 20–40 mg quantities was placed on milipore filters fitted within a syringe and rapidly washed with 0.5 ml hexane in less than 20 seconds. The eluate was collected directly onto filter paper (9 cm diameter), which was allowed to air dry until the solvent had evaporated and was weighed to the nearest 0.05 mg. The findings depicted in Fig. 1 show that for the three insect-pollinated species investigated, pollenkitt represented 3.6–5.5% of the pollen weight, which falls within the common range cited

above for whole-pollen ether extracts. For the single wind-pollinated grass species we examined (Fig. 1), however, we detected no pollenkitt; more refined measurement methods may be required to pick up the smaller amounts of pollenkitt deposited on anemophilous pollen (Hesse 1980).

The suggestion that pollenkitt is the source of pollen odor (Knoll 1930, Porsch 1956, van der Pijl 1964, Faegri and van der Pijl 1979, Zandonella et al. 1981, Buchmann 1983, Bernhardt 1986) was initially substantiated in the diversity of chemicals reported in pollenkitt of a few species (Wittgenstein and Sawicki 1970, Egorov and Egofarova 1971 in Stanley and Linskens 1974, Roberts et al. 1979) and in pollenkitt-containing extracts of pollen (Stanley and Linskens 1974). Subsequently, Dobson (1988) conducted a chemical survey of pollenkitt oils in 69 angiosperm species (mostly insect-pollinated) using thin layer chromatography. She found wide variation between species in terms of the quantity and diversity of neutral lipids, which encompass compounds typically found in plant odors, including isoprenoid compounds, fatty acid derivatives, and benzenoid compounds, as well as mono-, di- and triglycerides, and pigments. Suggestive patterns in the detected compounds followed taxonomic lines, with congeneric species showing strong similarities in their compositions, a finding that has been supported in subsequent analyses of pollenkitt involving a few species (Houston et al. 1993, Pham-Delègue et al. 1994). These studies paved the way for investigations of pollen volatiles (Dobson et al. 1990, 1996; see the section below) and for the confirmation that volatiles contained in pollenkitt are the same as those in pollen headspace.

Evidence that pollenkitt is the site of pollen odor emission was obtained by Dobson et al. (1987), who used the fragrant pollen of *Rosa rugosa* (Rosaceae) as a model for comparing the volatiles in pollen headspace with those in the pollenkitt (washed from pollen with hexane) using gas chromatography. They found that all the main components in the pollen

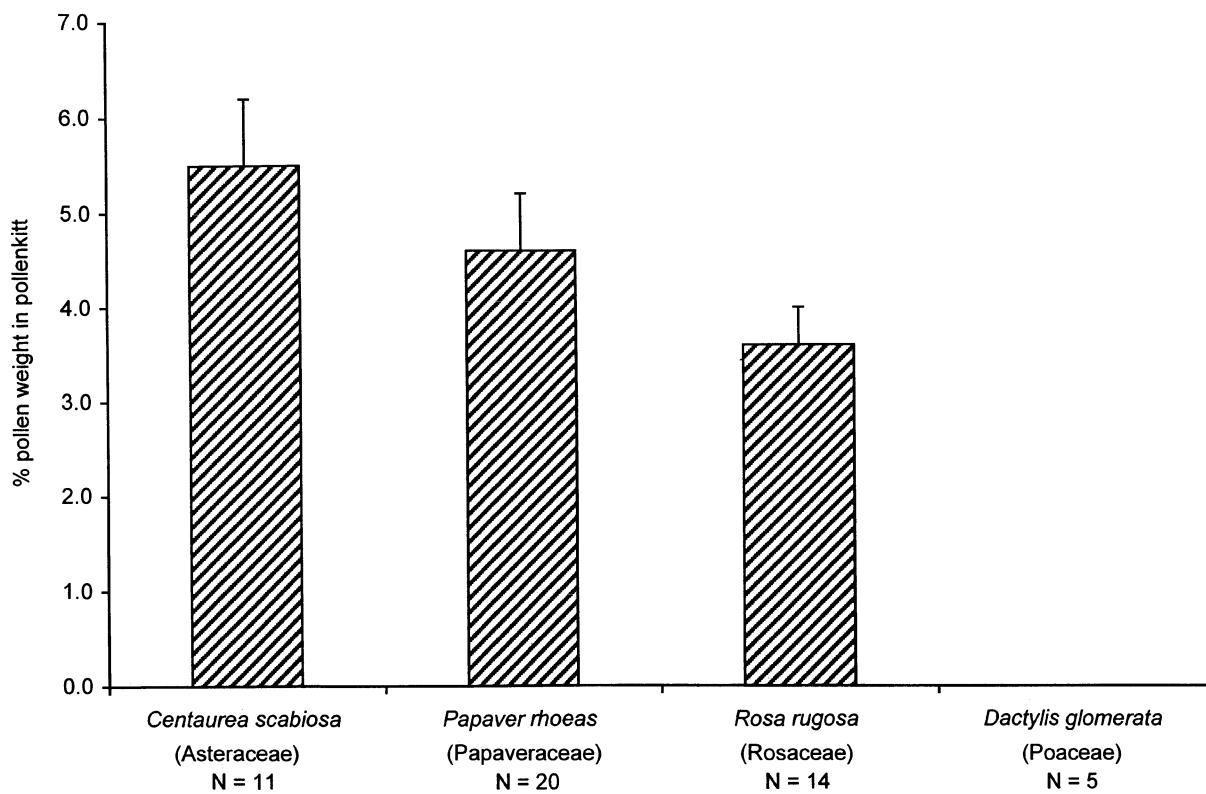


Fig. 1. Percent of pollen weight that is represented by pollenkitt in three entomophilous and one anemophilous species. No pollenkitt was detected in *D. glomerata*. Error bars represent ± 1 s.e.m.

headspace (Dobson et al. 1987, 1990) were also detected in the pollenkitt (Dobson et al. 1987, Dobson 1989), which confirms that pollen volatiles are indeed coming from the pollenkitt (Table 1). In the analysis, the relative proportions of individual volatiles were often different between the headspace and pollenkitt-hexane samples (Dobson 1989), but this is expected since the headspace shows only the compounds released in the gaseous phase at the time of collection whereas pollenkitt extract shows the total volatiles contained. Subsequent studies of pollen odors have focused on the analysis of headspace samples, since they represent the mixtures of volatiles actually emitted into the air.

In their attempt to open the field of pollen odor studies and lay a foundation in the chemical identification of pollen odor compounds (Dobson et al. 1987, 1990, 1996; Bergström et al. 1995), Dobson and collaborators developed methods to collect and ana-

lyze pollen volatiles by adapting headspace techniques used for flowers (Dobson 1991). The study of pollen odor has been especially challenging due to the low rate of volatile release from pollen compared with other scent-producing floral parts. This slow volatilization may be a consequence of the presence of other lipids in pollenkitt, such as mono-, di- and triglycerides, which can retard the emission of volatiles. However, a gradual release of the finite quantity of pollen volatiles, contained in pollenkitt when it is deposited on the pollen grains prior to anther dehiscence, could be of clear adaptive value to the plant by ensuring that pollen continues to produce odor until it is picked up by pollinators. To overcome the limited emission of volatiles, which reduces the success of volatile detection and identification using gas chromatography and mass spectrometry, 50–200 mg quantities of hand-collected pollen are used for headspace sampling, and volatile collection is carried out over

24–48 hours. It is important that special care be taken to minimize contaminations of the sample, which can obscure the detected volatiles. Furthermore, to identify odors coming specifically from pollen, hand-collected pollen is preferred over the more readily obtained bee-collected pollen (Collin et al. 1995), which may contain various pollen contaminants and show alterations in volatile compositions due to added bee secretions and nectar.

Pollen odor chemistry

To date, pollen headspace volatiles have been analysed in over 15 species from 10 families, but in only 12 species, representing 11 genera and 7 families (all entomophilous, see Table 2), have the collected volatiles been of sufficient quantity for most of the compounds to be identified. In only 6 of these species (*Lupinus polyphyllus*, *Papaver rhoes*, *Ranunculus acris*, *Filipendula vulgaris*, *Rosa rugosa*,

Table 1. Volatiles detected in pollen headspace and pollenkitt hexane-wash of *Rosa rugosa*. For headspace, XX = “major” compounds detected in amounts $\geq 2\%$, X = “minor” compounds in amounts $< 2\%$ of total; for pollenkitt, X = detected

Compound ^a	Pollen ^a	Pollenkitt ^b
Fatty acid derivatives		
Tridecane	X	
Pentadecane	XX	X
Heneicosane	X	
6-Methyl-5-hepten-2-one	X	
2-Undecanone	X	
2-Tridecanone	XX	X
Tetradecanal	XX	X
Hexadecanal	XX	X
Tetradecyl acetate	XX	X
Hexadecyl acetate	XX	X
Isoprenoids		
Geranial	X	X (?) ^c
Geraniol	X	X
Geranyl acetone	XX	X
Citronellyl acetate	XX	X
Neryl acetate	X	X
Geranyl acetate	XX	X
α -Farnesene	XX	X
Sesquiterpene a	X	
Sesquiterpene b	X	
Sesquiterpene c	X	
Sesquiterpene d	X	
Sesquiterpene f	X	
Sesquiterpene g	X	
Benzoids		
2-Phenyl ethanol	X	X (?) ^c
Methyleugenol	XX	X
Eugenol	XX	X
2-Phenylethyl acetate	X	X

^a List includes only compounds detected in pollen odor, and their relative amounts, in Dobson et al. 1990

^b Modified through reevaluation of data in Dobson et al. 1987

^c Detection questionable

Lycopersicon esculentum) have the pollen odor compositions been confirmed in repeated samplings (Dobson et al. 1987, 1990, 1996; Bergström et al. 1995; Dobson, Bergström, and Pressman, unpublished). The three major classes of compounds reported in floral scents, namely fatty-acid derivatives, isoprenoids, and benzenoids (Knudsen et al. 1993), are represented in pollen odor, but in amounts that vary among species (Table 2). In half the species all three chemical classes are represented, in the other half only two. Identification of volatiles reveals that each species has its specific pollen-volatile profile representing a unique mixture of compounds. In our studies, pollen odors vary in the number of detected volatiles from 5 to over 30. Most pollen odors are clearly dominated by specific volatiles, belonging to one or more chemical classes. The dominant compounds may comprise one or two volatiles (e.g. *Caltha palustris*, *Filipendula vulgaris*, *Lonicera caprifolium*, *Lupinus polyphyllus*, *Papaver rhoeas*, *Ranunculus acris*) or several volatiles (e.g. *Rosa rugosa*). Some pollen types have strong odors that are readily analyzed using headspace techniques (e.g. *Filipendula vulgaris*, *Papaver rhoeas*, *Rosa rugosa*), whereas others have more subtle odors (e.g. *Centaurea scabiosa*, *Cichorium intybus*, *Lonicera caprifolium*) that can be problematical in distinguishing their components from background noise and establishing their chemical identities. In summary, it can be concluded from these findings that pollen of each species emits its own characteristic mixture of volatiles and that there is no evidence for a general “pollen odor” common to different species.

Included in the analyses above of pollen odor is one species (*Lycopersicon esculentum*) that is buzz-pollinated, where the pollen remains inside the anther until it is released by the vibratory behavior of pollinating bees. Based on subjective evaluations of several buzz-pollinated species with povicidally dehiscent anthers, floral odors appear to arise mainly or exclusively from the anthers (Coleman and Coleman 1982, Buchmann 1983, D'Arcy et al. 1990), and the pollen is described as typically

dry and devoid of pollenkitt. However, SEM studies challenge this view by showing that in *Lycopersicon* (Solanaceae) the pollen is joined by tapetal fluid within the anthers and that in *Actinidia deliciosa* (Actinidiaceae) the amount of pollenkitt varies with anther age (King and Ferguson 1994), suggesting that pollen odor might vary in a similar temporal fashion. Accordingly, in young dehiscing anthers of *A. deliciosa* that contain abundant tapetal material, the pollen is covered with sticky pollenkitt and is probably more scented than in older anthers, where the tapetal material dries, yielding dry pollen with little pollenkitt. The emission of odors from pollen in these plants is supported by our detection of clear pollen odors in *L. esculentum* (Dobson, Bergström, and Pressman, unpublished) and by the behavior of pollinating bees on *A. deliciosa*, which, prior to landing, can recognize the individual flowers (male or female) that have dehiscing anthers (Goodwin and Steven 1993).

Comparative studies of odors from different floral parts indicate that the androecium contrasts with the rest of the flower (Dobson et al. 1990, Knudsen and Tollsten 1991, Sazima et al. 1993, Pichersky et al. 1994, Bergström et al. 1995), thereby showing parallels with visual patterns. The same conclusion is made for pollen as well. Pollen odor has been found to be chemically distinct from the whole-flower odor in all five species (included in Table 2) for which such comparisons have been made. Four of these species offer pollen as the only reward (Dobson et al. 1987, 1990, 1996) and one is nectariferous (Bergström et al. 1995). Differences between pollen and whole-flower odors are apparent in terms of three parameters (Table 3): (1) the total number of volatiles detected in each odor, (2) the number of volatiles that are common to both odors, and (3) the proportional representations in each odor of these volatiles in common. Based on this, contrasts in the odors range from subtle to strong. *Papaver rhoeas* showed the least contrasts, where the pollen and whole flowers differed mainly in terms of the proportional representation of certain major volatiles;

Table 2. General classes of volatiles detected in pollen headspace odors of all species for which compounds have been identified. The chemical classes are rated by their relative representation in each odor, where X = < 10%, XX = ≥ 10%, XXX = ≥ 25%, and XXXX = ≥ 25% of total volatiles. The chemical classification follows that in Knudsen et al. (1993)

Compound	Asteraceae	Caprifoliaceae	Fabaceae	Papaveraceae	Ranunculaceae	Rosaceae	Solanaceae
a <i>Cichoriium</i>							
b <i>Lupinus polyphyllus</i>							
c <i>Lonicera caprifolium</i>							
d <i>Scabiosa atropurpurea</i>							
e <i>Centauraea scabiosa</i>							
f <i>Onobrychis arvensis</i>							
g <i>Lupinus polyphyllus</i>							
h <i>Papaver rhoes</i>							
i <i>Calathia pubescens</i>							
j <i>Ranunculus acris</i>							
k <i>Vitis vulgaris</i>							
l <i>Rosa canina</i>							
m <i>Rosa rugosa</i>							
n <i>Malva sylvestris</i>							
o <i>Lycoptis esculentum</i>							
p <i>Solanum tuberosum</i>							
q <i>Philadelphus</i>							
r <i>Prunus avium</i>							
s <i>Malus domestica</i>							
t <i>Malus pumila</i>							
u <i>Malus sylvestris</i>							
v <i>Malus spectabilis</i>							
w <i>Malus baccata</i>							
x <i>Malus sylvestris</i>							
y <i>Malus sylvestris</i>							
z <i>Malus sylvestris</i>							
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ll <i>Malus sylvestris</i>							
mm <i>Malus syl</i>							

Table 3. Comparison between pollen and whole-flower odors in terms of total number of volatiles detected, number of volatiles common to both, and differences in the percent representation of volatiles in common, where X = weakly, XX = moderately, and XXX = strongly different. Data are shown for all five species investigated to date

Species	Total number of volatiles		Number of volatiles in common	Differences in % representation
	Whole flowers	Pollen		
Fabaceae				
<i>Lupinus polyphyllus</i> ^a	37	24	22	XX
Papaveraceae				
<i>Papaver rhoeas</i> ^a	26	33	22	X
Ranunculaceae				
<i>Ranunculus acris</i> ^b	30	5	5	XXX
Rosaceae				
<i>Filipendula vulgaris</i> ^a	20	10	10	XXX
<i>Rosa rugosa</i> ^c	24	29	17	XXX

^a Dobson et al. 1996

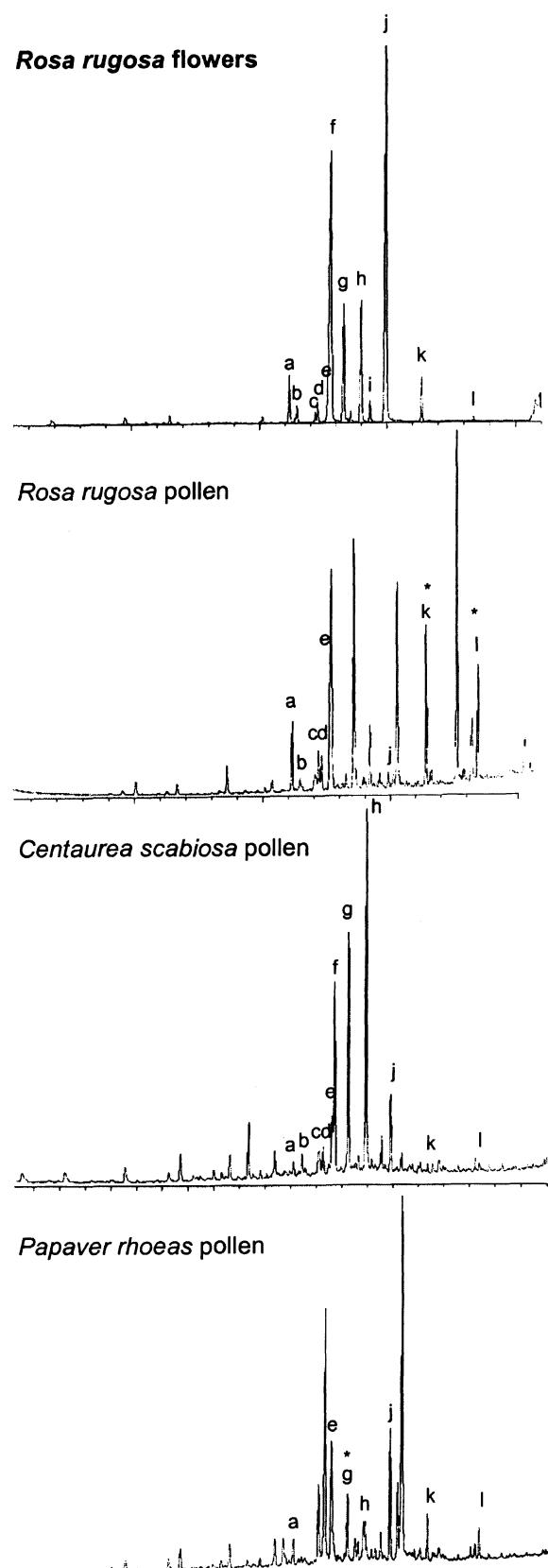
^b Bergström et al. 1995

^c Dobson et al. 1990

curiously, one third of the pollen volatiles were not detected in whole-flowers, but all were minor components in pollen odor and were probably at levels too low to be detected in the whole-flower odors. Contrasts were moderate in *Lupinus polyphyllus* in all three aspects. In both *Ranunculus acris* and *Filipendula vulgaris*, the contrasts were strong: pollen odor had markedly fewer volatiles, of which all were detected in whole-flowers but in often strikingly different proportions, with dominant compounds in pollen being only minor components of the whole-flower odor (and dominant volatiles in whole flowers being conversely often not detected in pollen odor). The most complex contrast, however, was presented by *Rosa rugosa*, which has a pollen odor composed of a similar number of volatiles as in whole flowers but where many (including major) volatiles are of distinct identity, and where volatiles in common were present in markedly different proportions.

The evidence is clear that pollen has an odor upon its exposure from the dehiscing anther, because it is already covered with pollenkitt. The pollenkitt substances are

formed inside the tapetal cells which, prior to anther dehiscence, break down and empty their contents into the anther loculus, where pollenkitt fills the exine cavities and covers the outer surface of the pollen grains (Pacini et al. 1985, Hesse and Hess 1993). Thus, pollenkitt – and pollen odor – is of sporophytic origin. However, the question remains as to what extent pollen odor can be modified through the uptake by pollenkitt of volatiles released from other flower parts during the time interval between anther dehiscence and pollen removal by pollinators. In *Rosa rugosa*, the volatile profile of pollen is strikingly different from that of the petals, but when pollen is left on the intact flower for over four hours after the anthers dehisce, greater quantities of mainly petal-originating volatiles may be found in the pollen odors than when the petals are removed upon anthesis. This suggests that the pollenkitt adsorbed the volatiles from the surrounding air laden with mainly petal odors (Dobson et al. 1990, Fig. 2). This effect could be of varying importance depending on the plant species and the time its pollen remains in the open anther before being removed.



To evaluate how uptake of environmental volatiles by pollen can differ among species, we conducted a study in 1988 where we exposed pollen of two other species to the floral volatiles of *Rosa rugosa*. We placed 100–130 mg freshly collected pollen in a Petri dish (10 cm diameter) lined with aluminum foil and surrounded it by a tight circle (15 cm diameter) of 20 newly opened intact *R. rugosa* flowers, elevating the pollen dish to situate it at the level of the petals. Pollen samples were left among the flowers for six hours and headspace volatiles were collected from the pollen using methods in Dobson (1991). As controls, we collected headspace odors from freshly-collected pollen of each species. *R. rugosa* flowers have an odor profile dominated principally by petal-originating monoterpenoid alcohols and 2-phenyl ethanol, and to a lesser degree by the androecium-originating volatiles eugenol and methyleugenol; *trans*- α -farnesene is a minor component (Dobson et al. 1990), as shown in Fig. 2. The adsorption of airborne *R. rugosa* volatiles onto pollen varied among the species, but certain compounds were adsorbed consistently by all three pollen types tested, namely 2-phenyl ethanol and *trans*- α -farnesene (Fig. 2). When *R. rugosa* pollen, which has a strong odor that overlaps little in chemical composition with that of the petals, was exposed to the flowers, its volatile profile was modified by the adsorption of small quantities of some petal volatiles, but this uptake was

Fig. 2. Gas chromatograms of pollen odors following pollen exposure to intact flowers of *Rosa rugosa* for 6 hours. *R. rugosa* pollen was exposed by leaving pollen on flowers; *C. scabiosa* and *P. rhoes* pollen was exposed by placing pollen in dishes surrounded by *R. rugosa* flowers. Lettered peaks indicate only volatiles present in odors of *R. rugosa* flowers, with an asterisk marking those also present in the pollen's own odor: *a* citronellyl acetate, *b* neral, *c* neryl acetate, *d* geranial, *e* α -farnesene, *f* citronellol, *g* nerol, *h* geraniol, *i* phenyl methanol, *j* 2-phenyl ethanol, *k* methyl eugenol, *l* eugenol. All other peaks correspond to volatiles detected exclusively in pollen; for identities, see Dobson et al. 1990, 1996

selective, since the three dominant monoterpene alcohols citronellol, nerol, and geraniol, were not detected. Pollen of *Centaurea scabiosa* (Asteraceae), which has a weak odor but is very sticky and seems to have abundant pollenkitt, changed markedly in its odor after exposure to the flowers. The prominent monoterpenoid alcohols (citronellol, geraniol, and nerol) were taken up the most, followed by *trans*- α -farnesene and 2-phenyl ethanol; several other compounds were adsorbed in minor quantities. Finally, pollen of *Papaver rhoesas*, which has a pronounced odor dominated by long-chain (C_{17} and C_{19}) hydrocarbons, showed at most only a moderate uptake of *trans*- α -farnesene, 2-phenyl ethanol, and possibly nerol (which is also a component of its own odor), and minor uptake of geraniol, methyleugenol, and citronellyl acetate. One trial was also made with pollen of the wind-pollinated *Dactylis glomerata* (Poaceae), but the odor sample collected was very weak and no volatiles could be conclusively identified; the low uptake could well be a reflection of the small quantities of pollenkitt on its pollen grains.

From our findings of the uptake of environmental volatiles by pollenkitt, we can conclude that: (1) pollen species vary in the amount and kind of volatiles adsorbed from the surrounding air, and this seems to be influenced by both the amount and chemistry of the pollenkitt; (2) volatile adsorption is selective, where certain volatiles are taken up more consistently across species than others and the amount of each volatile adsorbed is not necessarily correlated with its relative abundance in the air.

Pollen odor in relation to mode of pollination

If a primary function of pollen volatiles is to enhance pollination by pollen-foraging insects, one would expect flowers pollinated by nectar-seeking animals or by wind to have less prominent and distinctive pollen odors. Knoll (1930) pointed to pollen odor as playing an important role in attracting pollinator insects,

based on the observation that one finds very strong smelling pollen in various insect flowers in contrast to typical wind flowers. Strong pollen odors have in fact been described mainly in plants pollinated by pollen-feeding insects, especially beetles (Porsch 1956), such as *Lithocarpus densiflorus* (Fagaceae) and Cycadaceae (Faegri and van der Pijl 1979). However, Porsch (1956) argued that since pollen of wind-pollinated plants is equipped with pollenkitt, it too must have characteristic odors. He pointed out that several wind-pollinated species have prominent pollen odors (e.g. species in Cyperaceae) and that interestingly, most of these are also visited by pollen-feeding insects. Porsch provided numerous examples of beetles, as well as flies and bees, observed feeding on pollen from plants typically considered to be wind-pollinated, suggesting that pollen odor, alone or together with color, attracts the insects.

To determine if pollen odor is correlated with pollination mode, and the need to attract pollinators, von Aufsess (1960) compared the odor of pollen from plants depending on wind, insects, or birds for pollination using both subjective human evaluation and discrimination by honey bees in learning trials. In subjective evaluations, pollen of 13 anemophilous species (mainly Poaceae) had weaker odors that were also of a completely different quality compared to entomophilous pollen, suggesting that there are major differences in the types of volatiles present. However, anther scents in anemophilous plants were most often stronger and qualitatively different from inflorescence odors, but it is not clear how much of this contrast is due to pollen. In our studies of pollen headspace we examined four anemophilous species, *Pinus sylvestris*, *Betula verrucosa*, *Quercus robur*, and *Dactylis glomerata*, but in all cases the volatile profiles were very weak and further study is needed to identify the compounds.

Furthermore, headspace odors from anemophilous flowers suggest that male flowers may have greater proportions of α -methyl ketones and alcohols than either the corresponding

female flowers or animal-pollinated species. In our study of *Rumex acetosa* (Polygonaceae), which is dioecious, volatile profiles of the male and female flowers were similar except for the exclusive detection of α -methyl ketones and alcohols in the male ones (Table 4). A similar trend is apparent in the wind-pollinated *Cycas rumphii* (Cycadaceae) (Pellmyr et al. 1991), where male-cone odors displayed a greater preponderance of α -methyl ketones and alcohols than female-cone odors, which had more aliphatic esters (Table 4). As a point of emphasis, no α -methyl ketones or alcohols were detected in the odors of male cones of four simultaneously studied insect-pollinated cycads (Pellmyr et al. 1991). While these findings are difficult to extrapolate to pollen, since it is not known what contribution pollen is making to these whole-flower or cone odors, our studies of pollen headspace in entomophilous species lend some support to the proposed connection between α -methyl ketones and anemophily, or at least decreased entomophily. Indeed, in *Filipendula vulgaris* the pollen odor is strongly dominated by 2-heptadecanone, and the flowers, which attract only few insects, have been

suggested to be partly or mainly wind-pollinated (see Dobson et al. 1996). We have also detected α -methyl ketones in pollen odor of the bee-pollinated *Rosa rugosa*, which has a complex pollen odor that includes among its major components two α -methyl ketones (Dobson et al. 1990), of which one, and possibly both, deter landing by pollinating bumble bees when tested alone (Dobson et al. 1999). However, the pollen odor is also dominated by volatiles strongly attractive to bumble bees (e.g. eugenol), which appear to overshadow the deterrent effect of the α -methyl ketones. Taken together, these findings tentatively suggest that a major presence of α -methyl ketones and alcohols in flower and pollen odors may be associated with anemophily, or with reduced entomophily if these compounds are not counteracted by the presence of volatiles that are attractive to insects.

Comparative studies of pollen odor in plants pollinated by different animal groups are few, but they reveal patterns that are associated with the occurrence of pollen-feeding. Based on subjective human evaluations, species relying on pollen-feeding insects had

Table 4. Percent representation of different compounds in headspace volatiles from female and male flowers or cones of two wind-pollinated plants

Compound	<i>Rumex acetosa</i> (Polygonaceae)		<i>Cycas rumphii</i> ^a (Cycadaceae)	
	Female	Male	Female	Male
Fatty acid derivatives				
“green-leaf” (<i>cis</i> -3-hexenyl acetate, <i>cis</i> -3-hexenol, hexanol)	21.3	1.2	0.2	0.5
α -methyl ketones	—	19.2	4.2	15.8
α -methyl alcohols	—	14.4	25.6	51.4
esters			69.4	32.1
Isoprenoids				
monoterpene	28.1	15.8	0.5	—
sesquiterpenes	47.2	46.8		
irregular terpenes	1.8	1.1		
(6-methyl-5-hepten-2-one)				
Benzoids				
alcohols and esters	1.5	1.4		

^a Pellmyr et al. 1991

stronger pollen odors than did bird-pollinated plants in 22 of 24 pair-wise subjective comparisons (von Aufsess 1960). In the pollenkitt survey by Dobson (1988), the few species that are pollinated by animals seeking rewards other than pollen (i.e. Lepidoptera and hummingbirds) tended to have a pollenkitt with relatively few constituents compared to bee-pollinated species. Furthermore, human evaluations showed that pollen odor in entomophilous plants was qualitatively different from the petal odor in 15 of 17 species and in most cases was stronger, whereas in bird-pollinated plants it was qualitatively different in only 4 of 20 species and was equally often stronger as weaker. This more pronounced olfactory contrast of pollen against the remaining flower in entomophilous than in ornithophilous species was supported in bee training experiments. Honey bees clearly distinguished between the stamens and flowers of *Oenothera fruticosa* and between the pollen and flowers of a rose hybrid, but showed little or no ability to discriminate stamens against flowers in 5 of 6 ornithophilous species. This suggests that pollen scent in bird-pollinated plants is either too weak or is not sufficiently different from the rest of the flower for detection by bees.

While there appears to be a general trend for pollen odor to differ in association with pollination mode, the evidence is spotty and research is needed to get a more definitive picture of how pollen-vector type may have influenced the evolutionary selection for pollen odor emission. Comparative chemical studies of pollen odor between plants differing in pollination mode and of odor patterns in flowers presenting special cases of insect pollination, would help clarify to what extent pollen odor has evolved in association with the need to attract pollen-foraging pollinators. Some examples of insect-pollinated species that would be of special interest for investigation include those that have heterantherous flowers with fodder and fertilizing pollen (Endress 1994b), where fodder pollen might be olfactorily more attractive than fertilizing

pollen; nectarless species with rewardless female flowers that appear to mimic male flowers possibly through odor (Ågren and Schemske 1991); and cryptically dioecious species with poricidal anther dehiscence, where female flowers producing non-functional pollen are visited by pollen-foraging bees, suggesting that pollen odor is similar in both male and female flowers (Levine and Anderson 1986, Cane 1993, Knapp et al. 1998).

Effects of pollen odors on animals

Pollen is an important food (especially protein) source for many flower-feeding insects, but studies investigating how pollen stimuli, mainly visual and olfactory, influence the location and selection of pollen by insects are few (Dobson 1994). The distance at which pollen odors operate undoubtedly depends on both plant and insect species involved, but given that pollen odors are generally quantitatively weak compared to those of the whole flower, they are probably perceived by insects mainly at short distances, just prior to and after alighting on the flower. Furthermore, individual volatiles in the odor may be perceived at different distances within this close range, as determined by their volatility. At pre-alighting distances, pollen odors could allow insects both to recognize different pollen host-plants and to assess the amount of pollen reward, whereas after alighting they could help guide insects to the pollen and provide further species-specific recognition cues, including some that stimulate feeding. At any stage of plant selection, other stimuli (visual, tactile, gustatory) may act in concert with olfactory cues, with the relative importance of each depending on the particular insect-flower interaction in question (Dobson 1994). In honey bees and bumble bees, odors are learned more rapidly and with a greater retention than colors, and evoke stronger discrimination between flowers (e.g. von Frisch 1923, Kugler 1943, Menzel 1985, and see Dobson 1994), which places them higher in the hierarchy of floral stimuli that modulate flower and pollen selection.

By having pollen with a distinct odor, a plant gains both an additional species-specific identification label by which bees and other flower-visiting insects can discriminate it from other species and a means of advertising the presence of pollen rewards. The pollination-related advantages conferred on a plant from having pollen odor could translate into both greater foraging efficiency for the insect, through more efficient location and selection of food plants, as well as in increased fitness (especially male fitness) for the plant, through enhanced flower constancy, greater pollen export, and more effective pollen transport to stigmas.

Volatiles from pollen have been implicated in the host-plant selection of several pollen-feeding insects, with most evidence coming from studies of bees. Among non-bee insects, pollen odors appear to be attractants to plant-specialist beetles, including the weevil *Bruchus pisorum* (Bruchidae) which feeds on *Pisum* (Fabaceae) pollen (Stephen L. Clement, pers. comm. 1993), *Zygogramma bicolorata* (Chrysomelidae) which feeds on pollen and other plant parts of *Parthenium hysterophorus* (Asteraceae) (Jayanth et al. 1993), and the pollen beetle, *Meligethes aeneus* (Nitidulidae), which feeds on oilseed rape pollen during both adult and larval stages (Charpentier 1985). Recent olfactometer studies show that adult *M. aeneus* are attracted to flowers by odors, including those from pollen, and that they prefer male-fertile over male-sterile plants both in their general attraction to plants during bloom and in their oviposition, which implies the involvement of pollen odor (Samantha Cook, pers. comm. 1999). A plant-specific thrips species appears to use pollen odor to recognize its host-plant pollen, whereby it inspects pollen grains without touching them prior to initiating any probing (Kirk 1985). In the sunflower moth *Homoeosoma electellum* (Pyralidae), which requires sunflower pollen during the larval stage, pollen volatiles physiologically affect virgin females by triggering them to initiate calling behavior earlier, to spend more time calling, and to have a higher

rate of egg maturation (McNeil and Delisle 1989), which allows the females to adjust their reproduction to the temporally and spatially variable availability of pollen. In addition, pollen odor contains a volatile oviposition stimulant that enhances the female's location of newly opened sunflower heads (Delisle et al. 1989). At least some of the same volatiles might be involved in the initiation of calling behavior and oviposition by the European sunflower moth *H. nebulosum*, where both behaviors are induced in part by pollen volatiles detected at a distance ≥ 3 cm (Le Metayer et al. 1993). A greater effect of volatiles might have been obtained had the distance to the odor source (i.e. pollen test sample) been shortened, which would have increased the moth's perception of any active chemicals with low volatility.

Bees clearly perceive pollen odors. Pollen volatiles may be required to elicit landing, as in foraging-naive bumble bees, where it is most effectively elicited by a combination of visual signals from the anthers and olfactory stimuli from the pollen (entomophilous only) (Lunau 1992). Kleptoparasitic *Nomada* (Anthophoridae) bees are more attracted to host nests containing pollen than to those without (Cane 1983). When offered a choice of pollen from different species, honey bees inspect each pollen dish by hovering above it, implicating that they are using odor and/or color to evaluate the pollen prior to landing (Schmidt 1982). Both von Frisch (1923) and von Aufsess (1960) were able to train honey bees to differentiate between odors of pollen and other floral parts, indicating that the distinctive odors of pollen within the whole-flower context are perceived by bees, even in flowers where the contrasts are subtle (e.g. *Papaver*).

Honey bees are olfactorily attracted to pollen (Doull and Standifer 1969, 1970, Doull 1974a, b) and can use pollen odor to discriminate between plant species when these are offered as either whole pollen (Levin and Bohart 1955, Doull and Standifer 1969) or pollen extracts (Doull and Standifer 1970), based on the different numbers of attracted

bees. Ether extracts of pollen, which contain pollenkitt and thus pollen odor compounds, are attractive to foraging honey bees and stimulate them to collect non-pollen materials that are otherwise avoided (Louveau 1959, Hügel 1962, Taber 1963, Lepage and Boch 1968, Hohmann 1970). Honey bees can be trained to olfactorily discriminate between pollen of different species (von Frisch 1923, von Aufsess 1960, Samantha Cook, pers. comm. 1999), and specific odors carried on bees returning to the hive play a central role in the recruitment of foragers to new pollen sources (von Frisch 1923). Pollen selection may be mediated in part by specific volatiles, given that chemicals attractive to honey bees, including unidentified sterol-related compounds (Louveau 1959, Hügel 1962) a carotenoid ester and a free fatty acid (Standifer 1966, Lepage and Boch 1968, Hopkins et al. 1969), and to pollen-specialist solitary bees (Dobson, unpubl.), have been isolated from pollen. Deterrent compounds might also influence pollen selection (Free 1970, Schmidt 1982), as suggested especially when bees actively avoid pollen or remove from their bodies any pollen incidentally picked up from flowers (Cazier and Linsley 1974, Hurd et al. 1980, Waller et al. 1984).

Pollen odor can present a more species-specific chemical signal than the whole-flower fragrance by including volatiles that are not commonly emitted by nonpollen floral parts or that are only minor constituents in whole-flower odors. This is exemplified in the high representation of particular defensive chemicals, such as the lactone protoanemonin in pollen odor of *Ranunculus acris* (Bergström et al. 1995), or of various compounds including α -methyl ketones in *Rosa rugosa* pollen (Dobson et al. 1990, 1999). Conversely, pollen odor can become a more species-specific signal through the absence of major volatiles emitted from other flower parts, which tend to be more common in flower fragrances in general, as occurs in both the aforementioned species and in *Lupinus polyphyllus* (Dobson et al. 1996).

Behavioral studies with pollen-specialist solitary bee species indicate that the species-specificity of pollen odor can be of key importance in pollen host-plant recognition. Dobson and her students have tested four pollen-specialist bee species to determine what floral stimuli bees use to initially recognize their host-plants and how a bee's search image is modified after foraging experience. They used multiple-choice experiments, where bees were offered a choice of different plant species in the form of floral or pollen odors, and where bee preferences were measured by their feeding-attempt responses. When offered colors only, all bee species showed an innate feeding preference for yellow; the yellow stimulus was also required for three species to exhibit responses to odors. When tested with odors, preference patterns varied among the species (Table 5). In the most narrowly specialist bee, *Chelostoma florisomne*, which restricts its pollen and generally nectar foraging to buttercups (*Ranunculus* spp.), foraging-naive females could recognize their host plant more effectively when offered pollen odors than floral odors, and subsequent studies found that pollen odor contains a key chemical used in flower recognition (Dobson, unpubl.). However, after gaining foraging experience in the field, bees relied more on floral odors, which operate at longer distances than pollen odors (≤ 1 cm was needed for responses to pollen volatiles). In a population of *Colletes fulgidus* that specializes with respect to pollen foraging but is more generalist in its nectar feeding, foraging-naive females could recognize their pollen host-plant when offered either floral or pollen odors, both of which have strong and quite similar scents to the human nose; following foraging experience, this bee also appears to depend more heavily on floral stimuli (Dobson 1987). The other two bee species studied, *Dasypoda hirtipes* and *Heriades truncorum*, are more broadly specialized at the plant tribe or family level and have proved to be more challenging in the attempts to determine what role pollen odor plays in their host-plant recognition. Indeed, prefer-

Table 5. Host-plant recognition by pollen-specialist bees when offered a simultaneous choice of flower or pollen odors from four species. Recognition is based on the display of significantly more feeding attempts towards pollen host-plant(s) than nonhost-plants. Parentheses indicate incomplete data sets; (–) = not tested. All data are unpublished unless indicated

Bee species	Flower odors	Pollen odors
1. <i>Chelostoma florisomne</i> (Megachilidae) host plants: <i>Ranunculus</i> spp (Ranunculaceae)		
1) foraging-naïve	no	yes
2) foraging-experienced	yes	no
2. <i>Colletes fulgidus longiplumosus</i> (Colletidae) ^a host plants: <i>Grindelia stricta</i> (Asteraceae)		
1) foraging-naïve	yes	yes
2) foraging-experienced	(yes/no)	(no)
3. <i>Dasypoda hirtipes</i> (Melittidae) host plants: Asteraceae: Cichorioideae		
1) foraging-naïve	(no)	(no)
2) foraging-experienced	no	(–)
4. <i>Heriades truncorum</i> (Megachilidae) host plants: Asteraceae		
1) foraging-naïve	yes/no	yes/no
2) foraging-experienced	(–)	(–)

^a Dobson 1987

ences by these bees to either flower or pollen odors have being equivocal or not apparent (Dobson, unpubl.). These comparative studies across bee species make it clear that floral odor parameters characterizing host-plant selection in one bee-flower association do not necessarily apply to others; each association must be examined within its own biological and ecological context.

The presence in pollen of unusual volatiles, possibly with defensive functions, may be an essential feature that allows pollen-feeding insects to become specialized on particular plants, where the species- or genus-specific volatiles can serve as key recognition stimuli in host-plant location and selection. This scenario is evident in the specialization of the solitary bee *Chelostoma florisomne* on pollen of *Ranunculus*, where newly-emerged bees rely on pollen odor, and more specifically on its major component protoanemonin, to recognize their host plants (Dobson, unpubl.). A similar situation is suggested for the beetle

Zygogramma bicolorata (Chrysomelidae) that is a specialist on *Parthenium hysterophorus* (Asteraceae), where the sesquiterpene lactone parthenin attracts the beetles and induces them to feed (Jayanth et al. 1993). While the exact location of parthenin in the pollen has not been identified, the beetle's response is elicited when parthenin is presented either as whole pollen or as an aqueous extract of pollen, which is obtained in a process that would also wash off the pollenkitt; this suggests that some parthenin is contained in the pollenkitt, where it could olfactorily attract the beetles.

Pollenkitt can contain diverse compounds covering a range of polarities (Dobson 1988), and pollen compounds recently identified as feeding stimulants might possibly reside in part in the pollenkitt. Phagostimulant chemicals in pollen have been implicated in feeding preferences (based on consumption) by honey bees for one pollen over another (Schmidt and Johnson 1984) and by the hover fly *Eristalis tenax* (Syrphidae) for whole pollen or pollen

extracts over extracted pollen (Wacht et al. 1996). A variety of compounds appear to be involved, given that pollen extracts of different polarities stimulate feeding in honey bees (Robinson and Nation 1968, Schmidt 1985) and in the western corn rootworm beetle, *Diabrotica virgifera virgifera* (Chrysomelidae) (Hollister and Mullin 1999). Feeding stimulants in the hoverfly consist mainly of amino acids (Wacht et al. 2000), which are contained in pollen parts other than pollenkitt. However, chemicals in sunflower pollen that are strongly phagostimulatory to the western corn rootworm are more diverse, comprising lipid and midpolarity compounds that include primary common nutrients (fatty acids, glycerides, amino acids) as well as secondary chemicals, such as phenolic polyamides and flavonols (Lin and Mullin 1999). Some of these probably occur in pollenkitt, and some might be of sufficient volatility to act as very close-range olfactory stimuli. When dealing with pollen odor compounds of low volatility, it can be difficult to distinguish whether they are perceived through the modalities of olfaction or gustation, and behavioral data must be interpreted with care. Host-plant discrimination by pollen-feeding insects, including the two Asteraceae-pollen specialist bees discussed above, might likewise involve very short-range pollen volatiles that are in quantities too small to be perceived at the distances used in behavioral experiments testing for insect responses to pollen odors (e.g. 1 cm), and that may consequently be detected only in feeding tests.

Through the distinctness of pollen odor against the whole flower, pollen volatiles could provide pollinators a means for assessing pollen availability, thus allowing the insect to forage more efficiently by restricting its visits to the most rewarding flowers. The use of pollen odor to locate flowers offering pollen is suggested in observations of solitary bees cruising or gnawing nearly open flower buds that contain already dehiscing anthers (Hurd and Linsley 1963, Linsley et al. 1963, Eickwort 1973). Its further use to quantify a flower's pollen rewards is implicated in honey bees

preferentially visiting flowers of *Papaver* with the greatest quantity of pollen (Ribbands 1949). Foraging bees are often reported to "inspect" flowers, where they briefly hover above a flower prior to either landing or flying on (e.g. Ribbands 1949, McNaughton and Harper 1960, Heinrich 1979, Zimmerman 1982, Dobson et al. 1999). These inspections could be crucial times when bees evaluate close-range cues, both visual and olfactory, that allow them to assess pollen availability. This is supported by the increase in inspection frequency with time of day, as food rewards become depleted and bees show more selectivity in the flowers they visit. In flowers where pollen is fully exposed (Ribbands 1949, Zimmerman 1982, Cresswell and Robertson 1994) or where pollen depletion is accompanied by obvious visual cues, including age-related changes in floral morphology (Pellmyr 1988), post-pollination color changes (Wainright 1978), or visible alterations in anthers (Zimmerman 1982), it is difficult to establish the extent to which bees are processing visual as opposed to olfactory cues during an inspection. Pollen odor cues are, however, strongly implicated in some flowers that have concealed pollen, where bees have been observed assessing rewards prior to selecting individual flowers with the most pollen whether or not flowers display visual cues associated with changes in pollen availability (Heinrich 1979, Haynes and Mesler 1984, Gori 1989, Harder 1990, Shelly et al. 1993). Allowing pollinators to assess pollen rewards might be a primary selective force driving the evolution of distinctive pollen odor in some species (e.g. *Lupinus polyphyllus*, see Dobson et al. 1996). Pollen odor as a means of assessing food reward may also explain the preference of the syrphid fly *Episyrphus balteatus* for oil-seed rape flowers in which the number of anthers was experimentally increased (Golding et al. 1999).

The strongest evidence for the use of pollen odor by bees for discriminating between flowers that have different amounts of pollen comes from behavioral field studies of pollen-foraging bumble bees on *Rosa rugosa* (Dobson

et al. 1999). By variously altering the androecia of flowers presented to free-flying bees on blooming bushes, it is clear that bumble bees rely more heavily on cues (both visual and olfactory) from the androecium than from the petals to distinguish between pollen-offering first-day flowers and pollenless second-day flowers, and furthermore, that pollen odor modulates landing and pollen foraging on first-day flowers. Indeed, addition of certain major pollen volatiles, namely eugenol and tetradecyl acetate, to flowers from which anthers had been removed significantly increased both landings and typical vibratory pollen-collecting behavior in bumble bees. Interestingly, not all components of pollen odor enhanced flower visitation, with the α -methyl ketone 2-tridecanone and geranyl acetate having slightly deterrent effects on landing frequency. While pollinators encounter the entire mixture of volatiles in a pollen odor, their responses appear to be governed by their differential perception of the individual chemicals and by any interactive effects, both additive and synergistic, among the volatile constituents. The findings that volatiles in pollen odor can include chemicals that have differing effects, both attractive and deterrent, on pollinators point to pollen volatiles as playing multiple roles in the biology of pollen.

Pollen odors in plant defense

The apparently ubiquitous occurrence of odor in pollen, its varying chemical composition, and its inclusion of insect repellents and antimicrobial compounds, suggest that pollen odor chemicals serve other purposes in addition to pollinator attraction. These other functions include, most importantly, the widespread need to defend the male gametophyte against destructive pollen-feeding animals and pathogens, as well as the occasional defense of the plant against competition from other species through the phenomenon of pollen allelopathy.

Volatiles that deter non-pollinator insects from feeding on various floral parts, including

pollen, may be located in different parts of the flower (Casida 1980, Mullin et al. 1991, Wells et al. 1993). The presence of some of these chemicals in non-pollen floral parts might reduce the need for plants to deposit deterrent chemicals in the pollen, including the pollen-kitt. Anthers may keep pollen-feeding insects at bay by containing repellent chemicals (Belcher et al. 1983, Rossiter et al. 1986, Fujimori and Ashihara 1993, Werner et al. 1995). Alternatively, attractive volatiles in sterile pollen of heterantherous flowers (Faden 1992) or in highly attractive food structures, such as staminodes (Bergström et al. 1991, Endress 1994b) may lure pollen-feeders away from the fertile pollen. For plants with documented defensive compounds in anthers, it would be instructive to extend chemical analyses to pollen to clarify whether the defense role is relegated to anther tissue rather than pollen, or whether pollen also contains some of the same defenses, which might compromise its attraction to pollinators. It seems probable that plants with exposed pollen that is readily exploited by non-pollinating insects would be under greater selection pressure to produce antiherbivore deterrents in pollen, as found in *Rosa rugosa* (see below, Dobson et al. 1990) and *Ranunculus acris* (with protoanemonin) (Bergström et al. 1995).

The α -methyl ketones detected in pollen odors (discussed in sections above) appear to serve in defense against both insects and pathogens. The two abundant α -methyl ketones in pollen odor of *Rosa rugosa*, namely 2-undecanone and 2-tridecanone (Dobson et al. 1990), are deterrent and even toxic to several insects (see Kennedy et al. 1991, Farrar et al. 1992, Marr and Tang 1992, Maluf et al. 1997), and some α -methyl ketones show anti-fungal activity (Cole et al. 1975). In *R. rugosa*, 2-tridecanone is also a deterrent to pollinating bumble bees (Dobson et al. 1999), suggesting that some defense volatiles are broadly acting. In this particular example, the presence of highly attractive chemicals in the pollen odor (i.e. eugenol, tetradecyl acetate) appears to overshadow the deterrent effects of defense

compounds on bumble bees and allows the flowers to still rely on bees for pollination while the pollen is protected against pathogens and possibly certain herbivores. The α -methyl ketone 2-heptadecanone that strongly dominates the pollen odor of *Filipendula vulgaris* may likewise be one reason why the flowers attract few insects. Wind-pollinated plants have no need to attract pollinator insects, and one would therefore expect defensive chemicals to be more prominent in their pollen odor, which may explain the suggestive higher incidence of α -methyl ketones in anemophilous male flowers as compared to either their female counterparts or entomophilous flowers.

Various other defense compounds have been isolated from pollen, and although many may be constituents of pollen odor, in only few cases has this been experimentally confirmed. Compounds of mid to high polarity in whole pollen or aqueous pollen extracts, but not pollen hexane extracts, from sunflower are involved in the deterrence of ovipositing females of the banded sunflower moth *Cochylis hospes* (Cochylidae), which lay eggs on bracts even though larvae feed on pollen, but it is not clear if pollen volatiles play a role (Barker and Grugel 1996). Hydroxycinnamic acid-spermidine amides, which are located mainly in the pollen exine (and pollenkitt?) and are thought to function in defense against pathogens, have been isolated from genera in several orders by extracting pollen with methanol (Meurer et al. 1988), a process that would also remove the pollenkitt. Sesquiterpene lactones appear to have evolved as defense through their detergency to herbivores and activity against fungi and bacteria (Picman 1986), and at least one has been isolated in pollen (Sukhada and Jayachandra 1980a, b), where it might be contained in pollenkitt (see section above). It seems probable that sesquiterpene lactones are more widespread in pollen, considering that they occur in several angiosperm families and represent over half of the diverse sesquiterpenes that characterize all but one tribe of the Asteraceae (Langenheim 1994). Toxicity against fungi has been demonstrated in some pollen (Char and

Bhat 1975, Pandey et al. 1983, Tripathi et al. 1985), but the chemicals responsible for this activity remain to be isolated.

Volatiles in pollen odors may individually play more than one role in pollen biology. A diversity of essential-oil volatiles that include compounds reported in pollen odors and that are generally interpreted to attract pollinators might also serve as defense against bacteria and fungi (e.g. Morris et al. 1979, Knobloch et al. 1989, Kubo et al. 1995). For example, eugenol, a major pollen volatile in *Rosa rugosa*, both attracts a variety of insects and has known antimicrobial activity (Morris et al. 1979, Zaika 1988). From the insect perspective, pollen defense chemicals may become primary attractants to specialist pollinators or other specialist pollen feeders, as discussed in the previous section for the bee *Chelostoma florisomne* (Dobson, unpublished) or beetle *Zygogramma bicolorata* (Jayanth et al. 1993), and it is probable that other examples will be uncovered with more research. In the case of the sesquiterpene lactone parthenin, three different functions appear to be at play, including general defense, attraction of a specialist herbivore, and pollen allelopathy (Jayanth et al. 1993).

A rarely documented role for pollen volatiles is in the phenomenon of pollen allelopathy, where pollen of one species and has a detrimental effect on the pollen (and pollen germination) of other species, and whereby the species with allelopathic activity gains a competitive advantage and can increase in abundance (Murphy 1999). Pollen allelopathy has been demonstrated in three wind-pollinated species (two Poaceae and one Asteraceae) and agamospermous species of *Hieracium* (Asteraceae). Activity has been attributed to terpenoid and phenolic compounds that are extracted with water from pollen. In the grasses they are thought to be held in the intine and synthesized in the pollen cytoplasm (see Murphy 1999). In *Parthenium hysterophorus* (Asteraceae), however, the principal active compound is the sesquiterpene lactone parthenin (Sukhada and Jayachandra 1980a, b), which has been dis-

cussed above in the context of host-plant location by specialist herbivores and pollen defense, and which evidence suggests might in part be contained in the pollenkitt.

Summary and future research directions

The inclusion of volatiles in pollenkitt appears to have evolved under different selection pressures related mainly to pollinator attraction and pollen protection. However, while a variety of functions can be proposed for pollen odor, we need to obtain a big picture of the relative importance that these two forces have had in the evolution of pollen odor emission, such as through comparative surveys of plants with different modes of pollination. Studies of pollen odor in plants pollinated by wind and by nonpollen-feeding animals, or showing special cases of pollination mentioned in the text, would complement the available studies on plants pollinated by pollen-feeding insects; these would also clarify the occurrence of particular volatiles, including α -methyl ketones, in relation to pollination.

Evidence from the handful of studies conducted to date proposes several functions that individual volatiles (singly or in mixtures) may serve in the interactions between pollen on the one hand, and pollinators, pollen predators, and pathogens, on the other hand, and these are summarized in Table 6. These can be divided into activities that modulate the behavior of pollen-feeding animals or that interfere with the growth and survival of pathogens and other pollen. This list is tentative and will undoubtedly be added to over time. The field is wide open for investigation; one notable unrepresented area that deserves attention is the possibility that pollen volatiles might be involved in some aspects of pollen-stigma interactions. Experimental attempts to clarify the functions of specific pollen volatiles have demonstrated that each volatile may play multiple roles and that effects of the pollen odor chemicals on other organisms are complex and specific to the particular pollen-organism interaction in question. This empha-

sizes the need to evaluate each species-species interaction on a case-by-case basis.

In the pollen-organism interactions involving animals, species-specific pollen odors can enhance the animal's ability to discriminate between pollen – and flowers – of different species and consequently increase its pollen-foraging efficiency. The volatile cues would be learned during foraging and would be perceived as the insect is selecting among available pollen sources while still in flight. For pollen-specialist insects, species-, genus- or even family-specific chemicals in the pollen odors can serve as key recognition chemicals and thereby allow the insects to specialize on a particular species or taxonomic group of plants. Host-plant recognition by the insect, especially prior to it having any foraging experience, would occur prior to or after alighting on the flower, depending on the volatility of the key chemicals and the distance at which they can be perceived by the insect to trigger a behavioral or physiological response. It would be interesting to investigate if pollen odors in species visited by specialist bees have chemical characteristics, such as the presence of taxon-specific defense volatiles, that set them apart from others. More chemical studies of pollenkitt constituents and improved approaches to pollen odor analyses are needed to identify compounds of low volatility (e.g. sesquiterpene lactones in Asteraceae) that could be of value in the olfactory (i.e. pre-feeding) discrimination of pollen by both generalist and specialist pollen feeders. When pollen odor forms a distinct contrast with odors from other floral parts, it offers pollen-foraging insects a pollen-cue that they can use, through variations in its intensity, to assess the amount of pollen reward available in individual flowers during their in-flight selection of flowers to visit. If the insect is a pollinator, these three effects can result in greater foraging efficiency and flower constancy, which can translate into increased pollination and reproductive success for the plant. However, if the insect is a pollen feeder that does not participate in pollination, any features of the pollen

Table 6. Summary of ecological functions attributed to pollen odors in the interactions between plants (flowers) and other organisms. See text for specific examples

Function	Timing of function	Advantage to plant	Advantage to other
Modulation of animal behavior			
Host-plant selection by generalist pollen feeders	pre-alighting	yes/no ^a	yes
Host-plant recognition by specialist pollen feeders	pre- and post-alighting ^b	yes/no ^a	yes
Assessment of pollen reward in the flower	pre-alighting	yes/no ^a	yes
Defense of pollen against pollen feeders	pre- and post-alighting ^b	yes	no
Interference with growth and survival in non-animals			
Defense of pollen against pathogens (fungi, bacteria)	at all times	yes	no
Pollen allelopathy ^c	on stigma	yes	no

^a Depends on whether the pollen feeder is also a pollinator

^b Depends on the volatility of pollen odor chemicals and the distance at which they are perceived

^c Participation of pollen volatiles needs to be experimentally confirmed

odor that make it a more apparent target become a disadvantage to the plant. Comparison of feeding-deterrant chemistry in pollen and other floral parts (including anthers) would give insight into how defenses are spatially allocated within flowers and what impact this might have on pollination.

Interactions between pollen odor and non-animals seem to involve primarily interference, where pollen volatiles inhibit the growth and survival of pathogenic organisms. Pollen volatiles with activity would be present at all times in the odor, and the actual occurrence of such interference would depend on the encounter between pollen and pathogen and any conditions that would influence the pathogen's susceptibility to the volatiles. The inhibitory effects of pollen odor on pathogen growth is based mainly on circumstantial evidence. The antimicrobial activity of diverse floral – and pollen – volatiles has been demonstrated mainly *in vitro*, and conclusive evidence requires that pollen chemicals be tested *in vivo* against organisms that are ecologically significant to the plant species in question. It is also possible that defense volatiles might benefit bee pollinators that mass provision nest cells with pollen; chemicals that decrease pollen spoilage would result in higher larval survivorship and might be preferred as food sources by the next generation. With respect to the few document-

ed cases of pollen allelopathy, participation of pollen odor remains to be confirmed.

The field of pollen odor and elucidation of its adaptive significance is only at its beginnings and much more work lies ahead both for general investigations of pollen odor in relation to pollination and for specific studies of pollen odor chemicals in pollen-organism interactions. These will shed light on the selection pressures leading to the evolution of pollen odor emission and of the functions served by particular pollen volatiles.

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The ecology and evolution of visual pollen signals

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Abstract. By offering pollen and/or nectar as a food resource, angiosperms exploit flower visitors for pollen transport. Pollen thus acts not only as a means for transportation of male gametes, but also as a food reward for potential pollinators. Many findings provide compelling evidence that pollen acts, in addition, as a visual signal. The present contribution reviews several strategies that angiosperms have evolved to attract potential pollinators to the site of reward. We here consider evolutionary, ecological, sensory-physiological, and behavioural aspects of flower-pollinator interactions that are correlated with visual signals provided by pollen and pollen-producing organs, or imitations thereof.

Key words: Pollen, pollen colour, pollen pigment, colour pattern, UV-pattern, floral guide.

Introduction

Pollen is as old as the seed plants (*Spermatophyta*) themselves: a pollen “grain” is a haploid microspore that has matured through mitotic divisions. The primary and indispensable function of pollen is to transport the male gametes from staminate flower organs of one flower to pistillate flower organs to another conspecific flower. During this transport the pollen also provides essential protection: against desiccation, overheating, mutagenic

UV radiation, and damage by photo-oxidation and bacterial or fungal infections. Pollen pigments exhibit several protective functions (Asbeck 1954, Stanley and Linskens 1974, Swain 1977 as cited by McKey 1979, Dobson 1989, Milius 1998, Dafni, this volume). The primarily UV-absorbing, yellow colour of pollen originates from flavonoid pigments in the pollen wall (Harborne and Grayer 1993) absorbing mutagenic ultraviolet light and reflecting light of longer wavelength which may cause overheating. Flavonoids are common in pollen grains of seed plants, including the primarily wind-pollinated gymnosperms. Carotenoids seem to be associated with pollen and animal pollination (Barbier 1970, Stanley and Linskens 1974, Pacini et al. 1999). The presence of flavonoids in pollen of various families of the Angiospermae and the non-angiosperm taxa Taxaceae, Pinaceae, Cupressaceae, Taxodiaceae and Ginkgoaceae (Wiermann 1968, Stanley and Linskens 1974) suggests that this feature was already present in their common ancestor. Indeed, flavonoids have also been found in the spores of ferns and mosses (Wiemann 1968). If one accepts that flavonoid-containing pollen is a plesiomorphic character of the aforementioned taxa of the *Spermatophyta*, the data of Lloyd and Wells (1992) on the evolution of pollination systems,

when superimposed on a cladogram of the Spermatophyta, indicate that the first insect visitors of flowers probably encountered flavonoid-pigmented pollen with specific spectral reflection properties (Fig. 1).

Because the individual pollen grains are very small, with diameters from 0.25 mm (*Cucurbita pepo*) down to 0.005 mm (*Myosotis sylvatica*) (data from Grau and Leins 1968, Stanley and Linskens 1974, Heß 1983), considerable demands are made on the exine of the pollen wall, the main structure responsible for its protective function (see Hesse, this volume). The pollen grain must also provide the energy supply for germination, as well as sufficient amounts of the substances needed for outgrowth of the pollen tube (see Nepi & Franchi, this volume). With the change from anemophily to zoophily pollen takes on other functions: it adheres to the pollinators, serves them as a source of food, and attracts them by visual, tactile and chemical signals (see Dobson & Bergström, this volume). An individual pollen grain is too small to present a visual signal in

itself; where such signals are concerned, the crucial parameter is the display of relatively large amounts of pollen in the two thecae, each with two pollen sacs, of the anthers. The anthers themselves also have a signalling function. Furthermore, the tapetum cells of the thecae produce the pollenkitt which, once it has been incorporated into the exine of the pollen grains, also has a signalling function (Dobson 1989, Pacini 1997).

A review of the evolution and ecology of visual pollen signals must make reference to the other functions, because many aspects can be understood only in the light of the change, extension and loss of functions, and any discussion of adaptations of a particular function must take into account the retention of others.

Dafni and Giurfa (1998, 1999) pointed out that there is no consistent nomenclature for the visual patterns of flowers. In their terminology, which I adopt here, the three most common types of flower markings are distinguished as follows:

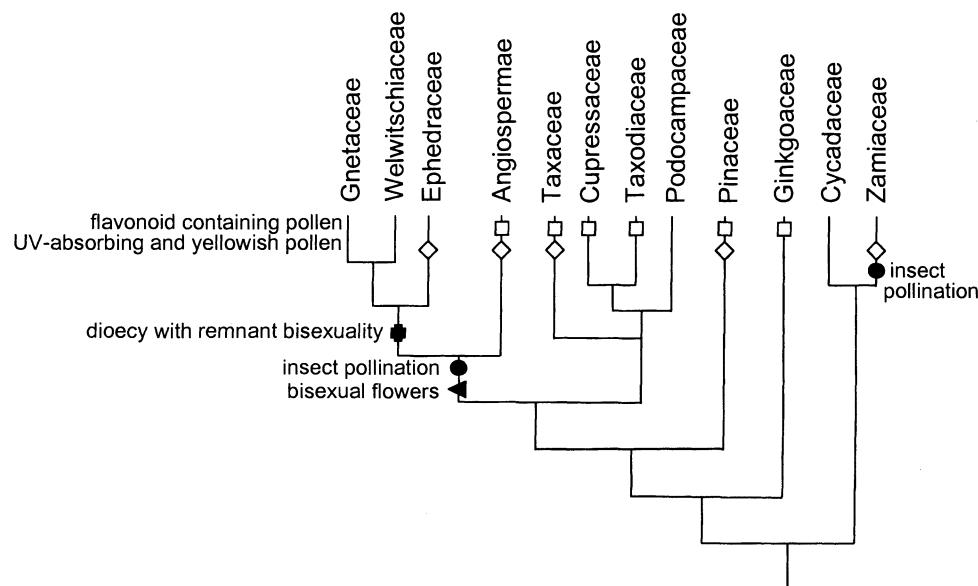


Fig. 1. Part of the cladogram of the taxon Spermatophyta (according to Chase et al. 1993). The filled signs indicate hypotheses concerning the branches in which some advanced character states evolved (according to Lloyd and Wells 1992): bisexual flowers, pollination by insects, dioecy with remnant bisexuality. The known occurrence of flavonoids in pollen (according to Stanley and Linskens 1985) and UV-absorbing and yellowish pollen (Lunau, unpublished) of some taxa is indicated by open symbols

1. Line, dot and dash markings, which act as guides and/or make the flower more attractive (Waser and Price 1985, Dafni and Kevan 1996).
2. More or less extensive, usually dark spots, which imitate the outline and the coloration of particular pollinators and attract the latter when they are looking for mating partners (Dafni et al. 1990, Johnson and Dafni 1998, Steiner 1998).
3. Imitations of pollen, anthers, stamens or a complete androecium, colour patterns sufficiently similar to substitute as signals for the actual pollen, anthers, stamens and androecium (Osche 1979, 1983b; Lunau 1996a).

The common terms "honey guide" and "nectar guide" are not relevant here, and the analogously constructed term "pollen guide" is not in common use. In this paper I shall employ the neutral "floral guide". This is defined to include not only patterns on the surface of flower parts but also structures in relief or fully three-dimensional.

Evolution of pollination by animals

Spore-eating insects existed over 300 million years ago, in the Carboniferous. Nowadays such insects help to distribute the spores of some recent species of myxomycetes, fungi and mosses (Gottsberger 1988). This is a matter of indiscriminate scattering of the spores and not a targeted transport such as occurs in the flowering plants, where animals mediate pollination by transferring pollen from staminate flower organs to pistillate organs, usually those of other conspecific individuals. Pollination by insects has evolved at least twice independently among the seed plants (Spermatophyta): in the cycads (Cycadales) and in the flowering plants (Anthophyta) (Norstog 1987, Pellmyr 1992a, Lloyd and Wells 1992) (Fig. 1). We can be certain that the insects initially used flowers as food resources, possibly attracted by the seed primordia, sugar-containing pollination droplets and/or pollen (Osche 1983b, Norstog 1987, Gottsberger 1988, Lloyd and Wells 1992, Kato and Inoue 1994). Presumably the food-seeking

insects preferentially visited those flowers that offered the desired resource. However, seed plants were originally unisexual; that is, nectar droplets and seed primordia were available only in pistillate flowers, pollen exclusively in staminate flowers. For flowering plants insects visiting either staminate or pistillate flowers selectively would have been a disadvantage, reducing the plant's reproductive success. It was not until bisexual flowers appeared that the food-seeking visitors could be effectively exploited as pollinators; now at every visit there was a chance of pollination, and at all but the first there was a chance of cross-pollination. Among the primitive flowering plants, the Annonaceae evolved pollination by beetles that used the flower tissue as a food resource (Gottsberger 1988), and some members of both gymnosperms (Gnetaceae) and angiosperms (Winteraceae) came to be pollinated by dipterans feeding on sugar-containing pollination, respectively stigmatic droplets (Gottsberger 1988, Lloyd and Wells 1992, Kato and Inoue 1994). In most of the primitive flowering plants (Magnoliaceae, Ranunculaceae) pollination is achieved by beetles, flies or bees that eat the pollen itself (van der Pijl 1960, Pellmyr and Thien 1986, Endress 1990, Bernhardt, this volume). The flowers frequently also provide nectar, and in some genera it is borne directly on the filaments of the anthers (*Cinnamomum*, *Laurus*, *Persea*; Lauraceae) (Bernhardt 1996) or on specialized nectar petals (*Helleborus*, *Nigella*; Ranunculaceae).

Because it must be put into an exposed position to allow wind pollination, and because of its protective pigments, pollen was predestined to become an attractant signal to visitors, especially in plants that had not evolved a conspicuous perianth. The first flower visitors were capable of detecting pollen colour signals, because trichromatic colour vision in insects is phylogenetically older than the habit of visiting flowers (Chittka 1996). Osche (1979, 1983a,b, 1986) recognized the pre-eminent significance of pollen as an attractant signal for flowering plants. From the original radially symmetric flowers with visible

androecium and pollen presented as food, zygomorphic flowers evolved in which the androecium was concealed and the food on offer was nectar; these flowers used mimetic signals as attractants, "signal copies" of pollen and stamens (Fig. 2). In comparison, nectar and nectaries are considerably less significant as attractant signals (Vogel 1993).

Behavioural responses of flower visitors to pollen signals

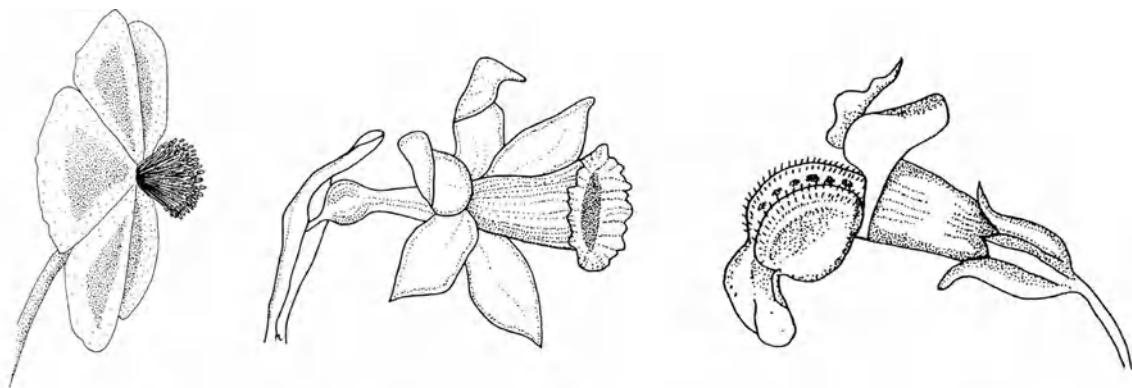
Many flower visitors eat the flowers' nectar and pollen. Among these are flies (in particular Bombyliidae, Syrphidae; Diptera), beetles (in particular Nitidulidae, Cerambycidae, Scarabaeidae; Coleoptera), a few butterflies (Heliconidae, Micropterygidae; Lepidoptera) (Erhardt and Baker 1990, Pellmyr 1992a), a few wasps (Masaridae; Vespoidea) and bees, both solitary (Megachilidae, Mellitidae, Andrenidae, Colletidae, Anthophoridae) and social (Apidae, Halictidae; Apoidea, Hymenoptera) forms (Kugler 1970). Bees also collect pollen as provisions for their larvae (Thorp 1979). Feeding on pollen has also been reported for certain marsupials (Marsupialia) and bats (Chiroptera) (Richardson et al. 1986, Herrera and del Rio 1998).

Pollen-eating insects are known to be able to orient to pollen and/or anthers by signals of various sensory modalities: tactile (Gack 1981), olfactory (Dobson 1988, this volume, Lunau 1992a), gustatory (Schmidt 1985, Wacht et al. 1996, Hansen et al. 1998) and visual (Lunau

and Maier 1995, Lunau 1996a). In the present context it is important to distinguish between innate behaviour, preprogrammed learning and associative learning. To signals that are innately recognized even inexperienced individuals of a species respond spontaneously, with a particular form of behaviour (Lunau 1996a). With preprogrammed learning an animal is predisposed to pay more attention to certain signals than to others, and can learn the former better and more quickly (Menzel 1985). Otherwise, individuals learn by experience, forming an association between specific characters and a reward.

Bees in particular have a highly developed capacity to learn visual stimuli (Menzel and Erber 1978; Menzel 1983, 1985). They acquire a preference for food plants with particular kinds of flowers, and for symmetrically formed flowers (Giurfa et al. 1995, 1996; Lehrer et al. 1995) over those with slight, fluctuating asymmetries, because the latter offer less nectar (Møller 1995). Bumble bees (*Bombus terrestris*), for example, in experiments with artificial flowers very rapidly and accurately learn to choose the one that provides a reward, on the basis of pollen signals (dummy anthers). In reciprocal training experiments, however, they can also learn the opposite behaviour: they can just as readily be trained to land on artificial flowers without pollen signals rather than those that have them, when offered a choice (Gack 1981). In these training experiments, the bumble bees were rewarded with a sugar solution, not with pollen. This situation re-

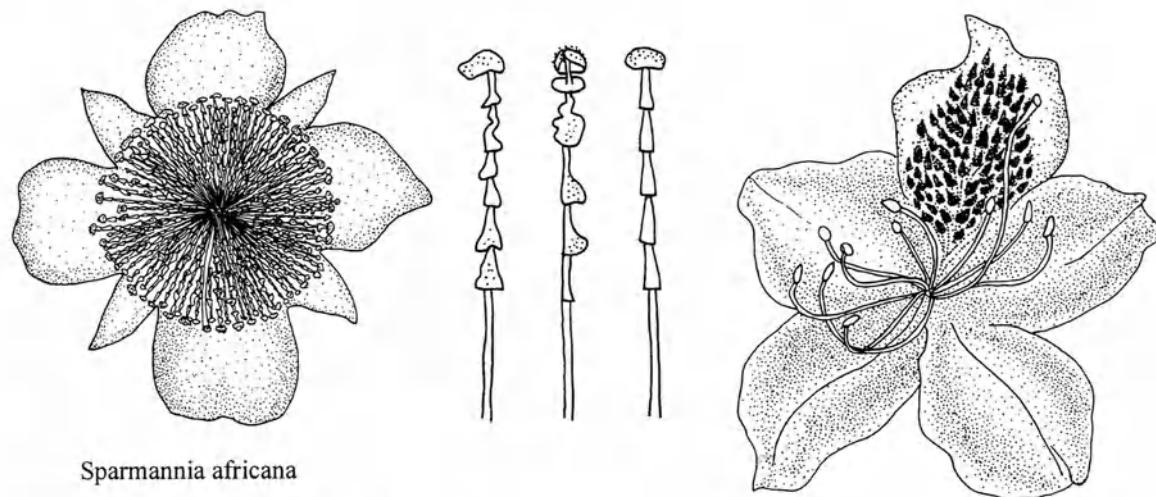
Fig. 2. Line drawings of bee-pollinated flowers: View from the side of the flower of *Helianthemum nummularium* (Cistaceae) with typical shape of the androecium when not mechanically stimulated. The shape of the inner corolla of *Narcissus pseudonarcissus* (Amaryllidaceae) resembles that of an androecium. The lower lip of *Mimulus guttatus* (Scrophulariaceae) resembles a single anther of supernormal size. The expanded androecium of *Sparmannia africana* (Tiliaceae) following tactile stimulation; enlarged: the nodular thickenings of the filaments of three stamens. The imitation androecium on the upward corolla leaf of the sternotribic *Rhododendron ponticum* (Ericaceae) flower marks the entrance to the nectary. The lower lip of the nototribic *Digitalis purpurea* (Scrophulariaceae) flower shows violet imitation anthers. The stigma of *Orobanche gracilis* (Orobanchaceae) mimics a single anther of a stamen. The yellow stamen patches on the petals of *Saxifraga stellaris* (Saxifragaceae) represent a uniformly attractive signal during the staminate flowering phase (left) in which also less attractive reddish pollen is offered and during the pistillate flowering phase (right) in which the anthers have fallen off. Some drawings modified from Osche (1983a)



Helianthemum nummularium

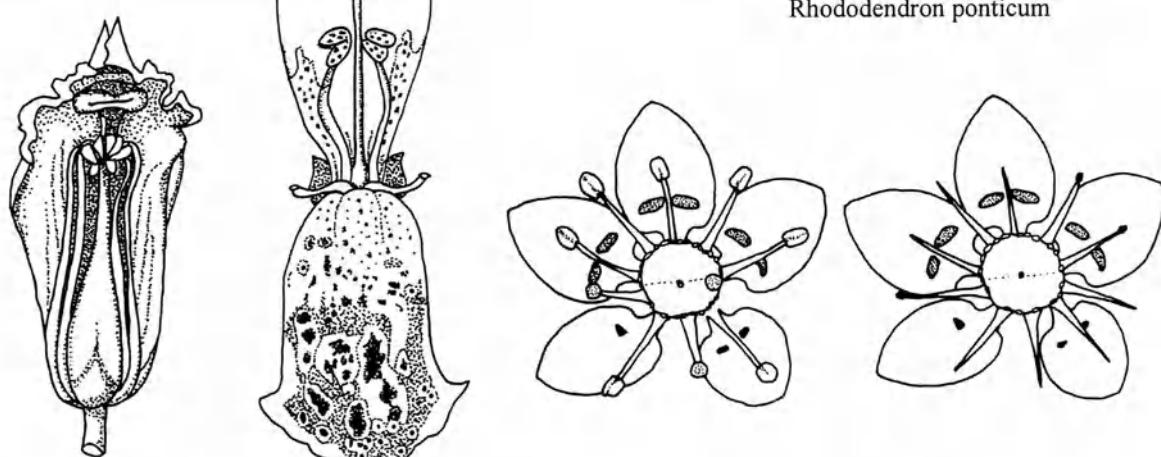
Narcissus pseudonarcissus

Mimulus guttatus



Sparmannia africana

Rhododendron ponticum



Orobanche gracilis

Digitalis purpurea

Saxifraga stellaris

sembles that in many real flowers, which advertise anther mimics and offer nectar as a reward.

Syrphid flies (*Eristalis pertinax*) in similar training experiments can also learn to land on targets either with or without pollen signals, but their maximal level of accuracy is lower (Lunau 1988). Honeybee and bumble bee workers with flower-constant behaviour temporarily restrict their visits to flowers of a single species (Waser 1986). Furthermore, during flower-constant foraging worker bees might prefer individual flowers that yield the most food; field studies have shown that bumble bee workers visited flowers of *Potentilla gracilis* with larger amounts of pollen more often than conspecific flowers with less pollen (Zimmerman 1982).

Although bumble bees and syrphid flies can be trained, by association with rewards, to discriminate among artificial flowers only on the basis of visual signals of pollen and stamens, newly emerged and untrained individuals of these species give innate, specific behavioural responses to the presence of visual pollen signals (Lunau 1993), which implies the existence of spontaneous preferences (Lehrer et al. 1995), an inborn search image (Menzel 1985) or innate neurosensory filter mechanism (Wehner 1981).

When syrphid flies (*Eristalis tenax*), with no experience of flowers, were tested in spontaneous-choice experiments, they responded to horizontally presented dummy flowers by extending the proboscis towards small spots of colour emitting yellow light, in the wavelength range from 510 nm to 600 nm (Fig. 3) (Lunau and Wacht 1994). Even slight admixtures of ultraviolet or blue light inhibit the proboscis response (Lunau and Wacht 1997). The neurosensory stimulus-filtering mechanism involved in this innate response is a "matched filter" (sensu Wehner 1987), one precisely tuned to the spectral reflection properties of yellow pollen of the natural food plants, which absorbs ultraviolet and blue light while reflecting green/yellow and red light (Fig. 3) (Lunau 1996a). After the proboscis has been extended,

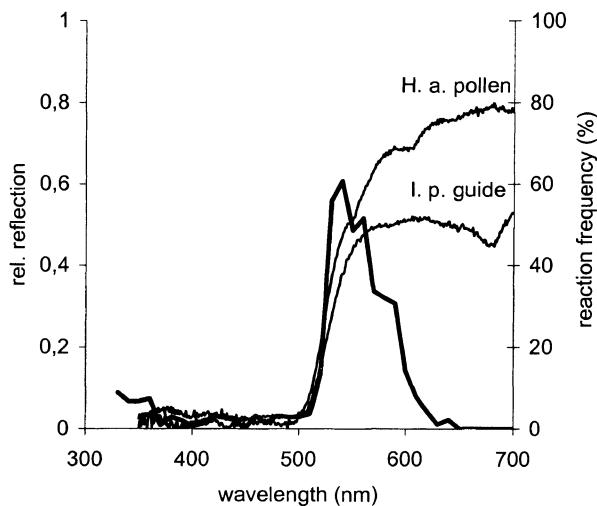


Fig. 3. Innate proboscis-extension response in the hoverfly *Eristalis tenax*. Frequency of flies extending their proboscis towards a monochromatic test light stimulus when passing a horizontal, white and UV-reflecting artificial flower (see Fig. 10). The test light was emitted by 4 small test screens each 2 mm in diameter. The intensity of the test light was 10^{13} quanta $\text{cm}^{-2} \text{ sec}^{-1}$. Ordinate: frequency of flies showing proboscis reaction towards the test light stimulus given as percentage of the number of tested flies ($n = 14810$). Abscissa: wavelength in nm. From Lunau and Wacht (1994)

a gustatory stimulus, the free amino acid proline, triggers ingestion of the pollen (Hansen et al. 1998). Remarkably, even after careful training *Eristalis* does not learn to give a proboscis response to colours other than the spontaneously chosen UV-absorbing yellow (Lunau 1988).

Results from some training experiments with bumble bees seem to indicate underlying colour preferences but with conflicting outcome: *B. terricola* prefers blue over white (Heinrich et al. 1977), *B. pennsylvanicus* prefers yellow over blue (Real et al. 1982), *B. terrestris* prefers blue over yellow (Smithson and Macnair 1996), yellow over orange (Chittka and Waser 1997). Therefore, only experiments with untrained bumble bees and unrewarded artificial flowers with dummy anthers are considered here to assess innate preferences. Flower-naive, untrained bumble bees (*Bombus*

terrestris, *B. lucorum*) orient to pollen signals before landing. When presented with vertical dummy flowers of a single colour, the bumble bees usually fly up to their edges, but when the flower has a colour pattern they may instead approach the differently coloured patch and touch it with the tips of the antennae (Fig. 4) (Lunau 1990). The landing is triggered by pollen scent emitted by the surfaces contacted during the antennal response (Lunau 1992a). Naive bumble bees are thus guided by innate means to the places on flowers that they then inspect more closely in search of food. Experienced and inexperienced bumble bees exhibit the antennal response also at floral guides of real flowers (Macior 1964; Lunau 1992a, 1996a). Patterns especially effective in eliciting the antennal response contain components of the visual appearance of an anther – for instance, two elongated ovals side by side. The size of the maximally effective dummy anthers is in the range of sizes of anthers in the natural food plants (Lunau 1991). The antennal response is most frequently elicited by a pattern comprising two spots, each representing a single anther spaced apart by the same distance that separates the tips of the two

antennae in their natural posture. Parameters of the colour pattern are crucial in triggering the innate antennal response. One component is the magnitude of the detectable colour contrast between artificial corolla and dummy anther, the other is the arrangement of these two colours. In spontaneous-choice experiments with a colour pattern comprising a dummy corolla and a dummy anther, bumble bees responded differently when the colours of corolla and anther were exchanged, even though the detectable colour contrast remained identical. The arrangement of the colours in the pattern is decisive: the bumblebees most often spontaneously perform the antennal response at the floral guide in the centre of the dummy flower when this area has the higher subjective colour purity (Fig. 5). The subjective brightness of the colours is irrelevant to the spontaneous choice. Naive bumble bees also do not exclusively rank the artificial flower colours according to their hue, but visited different blue, yellow and white artificial flowers in a mixed order of succession, indicating that distinct predominantly reflected wavelength regions are not preferred. Yellow, blue, white and simultaneously

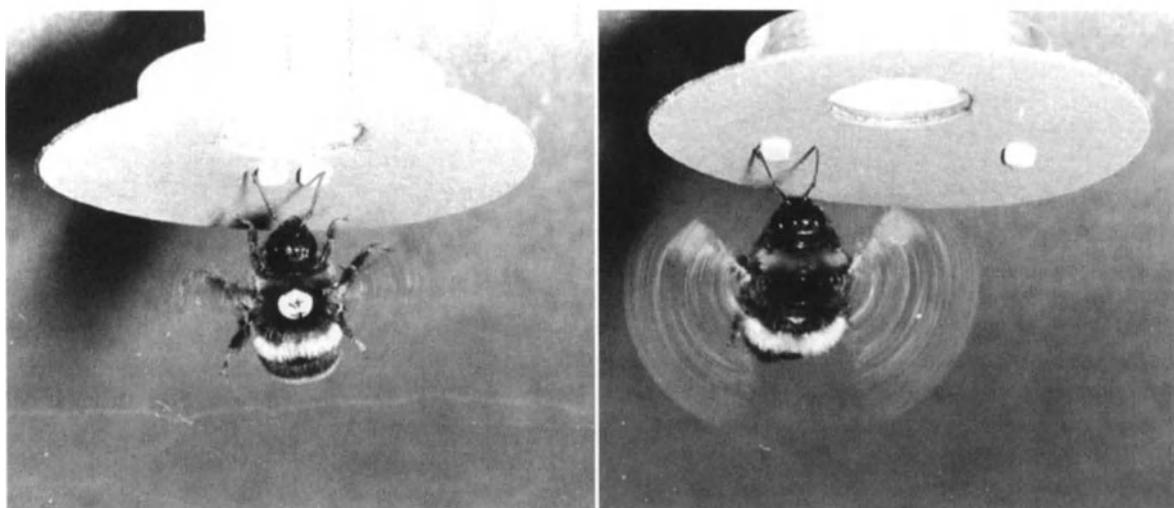


Fig. 4. Naive and untrained bumble bee worker exhibiting spontaneous antennal response towards the dummy anther of an artificial flower. Note the precision with which the antennal tips make contact with the dummy thecas when these are presented in different positions. Pollen odour is emitted from the hole in the central part of the artificial flower

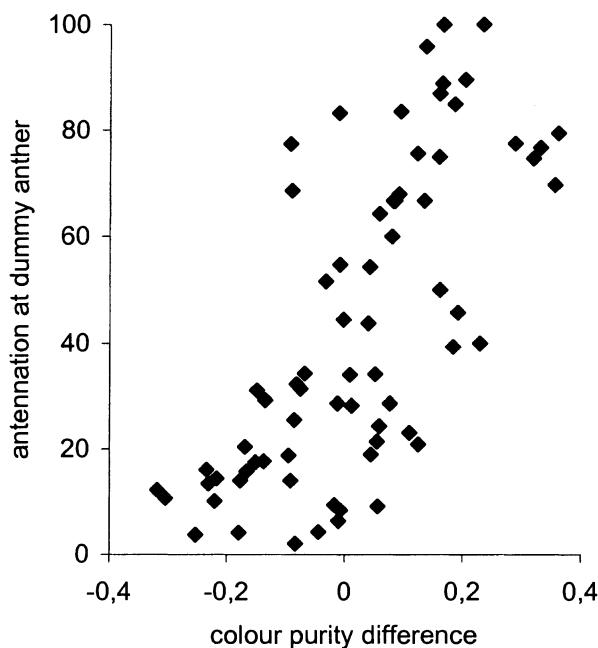


Fig. 5. Innate antennal response of flower-naive and untrained bumble bees (*Bombus terrestris*). Spontaneous choice behaviour following presentation of 70 different colour combinations of vertically arranged artificial corollas with dummy anther was tested. Ordinate: percentage of cases in which the approaching bumble bees showed an antennal response towards the dummy anther ($n = 7262$ approaches). Abscissa: difference between bee-subjective colour purity of the dummy anther colour and that of the artificial corolla colour. There is a significant positive correlation between the antennal response towards the dummy anther and the degree to which the dummy anther colour purity surpasses the artificial corolla colour purity. From Lunau et al. (1996)

UV-absorbing surfaces are all effective. The most effective predictor of innate colour preference is colour purity (Lunau 1990, Lunau et al. 1996). This neurosensory filter mechanism is matched to the colours of pollen and anthers, which themselves are distinguished by high colour purity (Lunau 1996a). In this context, it is remarkable that some flowers exhibit non-yellow floral guides, e.g. the violet dots of the floral tube entrance of *Digitalis purpurea* (Scrophulariaceae), the violet beard beneath the keel tip of *Polygala myrtifolia* (Polygalaceae) or the violet filamental hairs

of *Verbascum phoeniceum* and *V. nigrum* (Scrophulariaceae). All these structures have been described as imitation stamens by Osche (1979). Though without appropriate analysis of colour parameters, Kugler's (1935) observations of scouting bumble bees are consistent with these data, because the naive bumble bees he observed did not prefer a distinct hue, rather than intense and saturated colours both during orientation from some distance and at close range.

Signalling with pollen and stamens

At first glance the colour patterns of flowers seem extremely diverse. However, some of their components are much more common than others: UV patterns (Figs. 6, 7) (Kugler 1963, 1966; Ornduff and Mosquin 1969; Horowitz and Cohen 1972; Mulligan and Kevan 1973; Guldberg and Atsatt 1975; Biedinger and Barthlott 1993; Burr and Barthlott 1993; Chittka et al. 1994; Burr et al. 1995), the "ultraviolet bull's-eye" (Silberglied 1979), colour contrast between corolla and androecium (Daumer 1958, Kevan 1978, 1983; Osche 1979, 1983a,b), the predominantly yellow colour of pollen (Lubliner-Mianowska 1955; Daumer 1958; Osche 1979, 1983a,b), the reflection of relatively short wavelengths by peripheral parts of the flower (Kevan 1983), yellow flower centres (Kevan 1983) and gradients of centripetally increasing colour purity (Lunau 1992b, 1995, 1996a). All these colour patterns have to do with the signalling function of the pollen; they are compatible with a pattern generated by yellow, UV-absorbing pollen (or similarly coloured anthers or markings) in colour contrast to the corolla, which is probably the most common colour pattern of flowers (Figs. 6, 7). Comparable colour patterns are also formed by the disc florets of the inflorescences of many species of Asteraceae, in which the pollen retains its signalling function.

The visual signalling function of the androecium is documented by many findings. Pollen of primitive, wind-pollinated gymnosperms as a rule contains neither pollenkitt nor



Fig. 6. 1st row) Black-and-white photo and UV-photo of *Cucurbita* spec. showing UV-absorbing properties of pollen and androecium of a staminate flower and stigma mimicking in form and colour the androecium of a pistillate flower. 2nd row) Black-and-white photo and UV-photo of *Saintpaulia ionantha* showing the UV-absorbing properties of the anther walls. 3rd row) Black-and-white photo and UV-photo of *Colutea arborescens* showing the UV-reflection patterns of the flower with an imitation anther on the standard

carotinoids, whereas that of zoophilous plants has both (Bertrand and Poirault 1892, Euler et al. 1944, Lubliner-Mianowska 1955, Wier-

mann and Gubatz 1992, Pacini and Franchi 1993). The UV absorption of pollen (Figs. 8, 9) is based on the presence of flavonoids: di- and

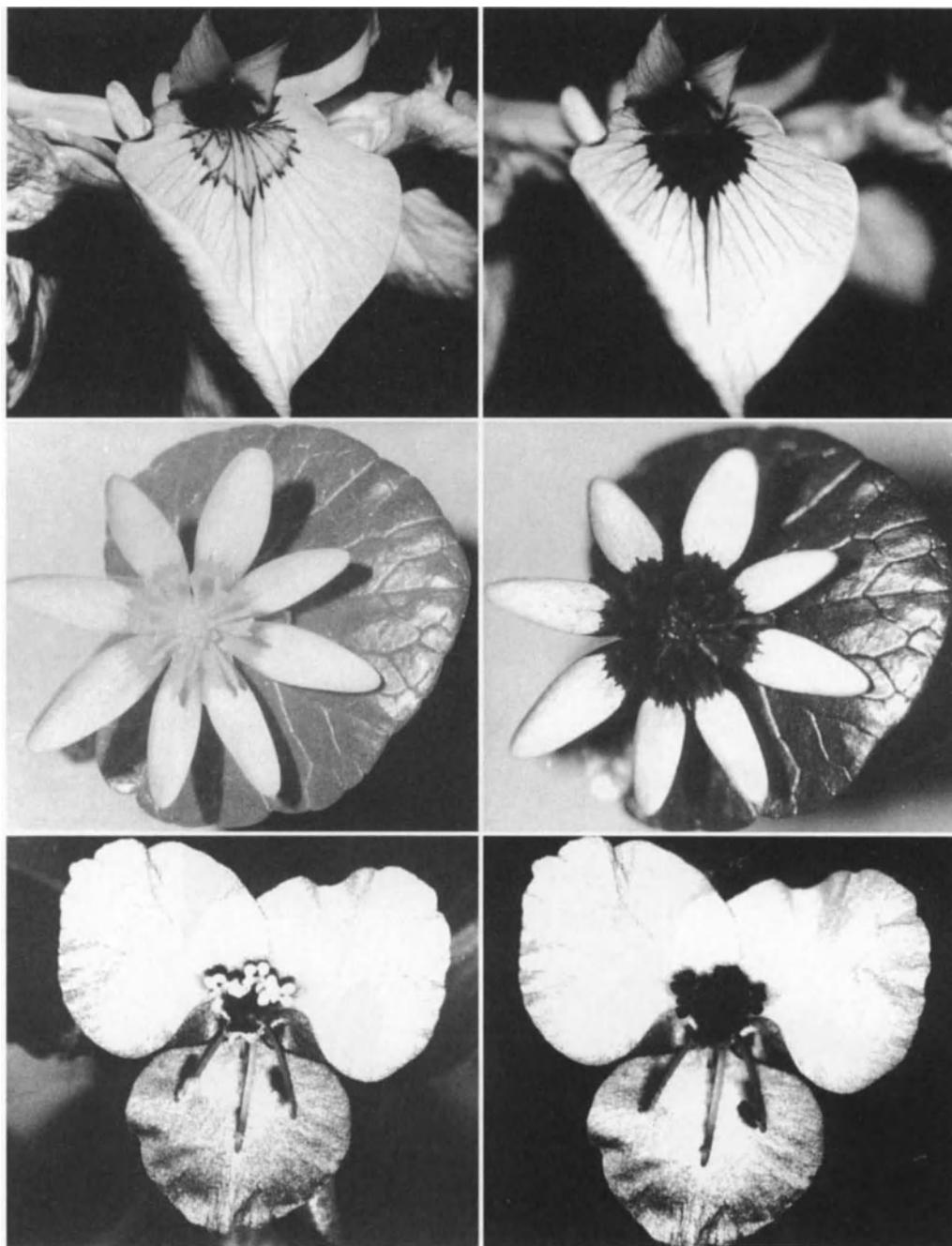


Fig. 7. 1st row) Black-and-white photo and UV-photo of *Iris pseudacorus* showing UV-absorbing properties of a yellow floral guide and a yellow corolla petal. 2nd row) Black-and-white photo and UV-photo of *Anemone fiucaria* showing UV-absorbing properties of pollen, androecium and basal areas of petals. 3rd row) Black-and-white photo and UV-photo of *Commelina coelestis* with UV-absorbing showy yellow staminodes

triglycosides of kaempferol, quercetin and isorhamnetin are common but of pale yellow tint, aglycosidic chalcones have an intense yellow colour (Wiermann and Gubatz 1992,

Brouillard and Dangles 1993, Harborne and Grayer 1993). The pollen of animal-pollinated plants that display it visibly is also an intense yellow (Figs. 8, 9) (Osche 1983b, Lunau 1995),

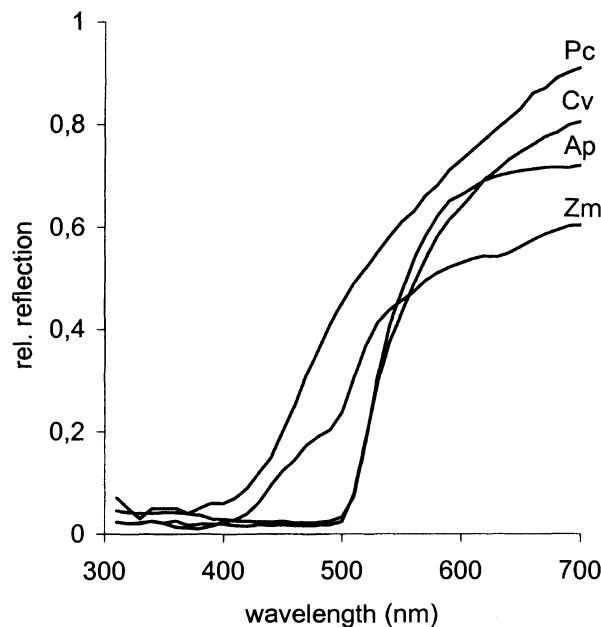


Fig. 8. Spectral reflection properties of pollen. The primarily wind-pollinated *Pinus cedrus* (Pinaceae) has yellowish UV-absorbing pollen (*Pc*). The secondarily wind-pollinated *Zea mays* (Gramineae) has yellowish pollen which absorbs ultraviolet light (*Zm*). The insect-pollinated *Chrysanthemum vulgaris* (Asteraceae) has bright yellow, UV absorbing pollen used as visual attractant (*Cv*). The insect-pollinated *Achillea ptarmica* (Compositae) has bright yellow pollen (*Ap*) similar to that of *C. vulgaris*

owing to the presence of carotenoids such as β -carotene, lutein, violaxanthine and antheraxanthine in the pollenkitt (Dobson 1988, Wiermann and Gubatz 1992). When pollen becomes secondarily concealed, its intense yellow colour often disappears (Fig. 9) (Pacini and Bellani 1986, Danner-El Hajami and Lunau, unpublished data).

In many species pollen and anthers are both involved in producing the colour pattern: the anthers are of the same yellow colour as pollen whether they are closed, releasing pollen or empty. In some cases the signalling function is taken over completely by the anthers. Then the pollen is invisible to the visitors, being hidden inside the anthers, and is released in portions through terminal pores. That the anthers have assumed this function is indicated by the intense yellow colour and also by their

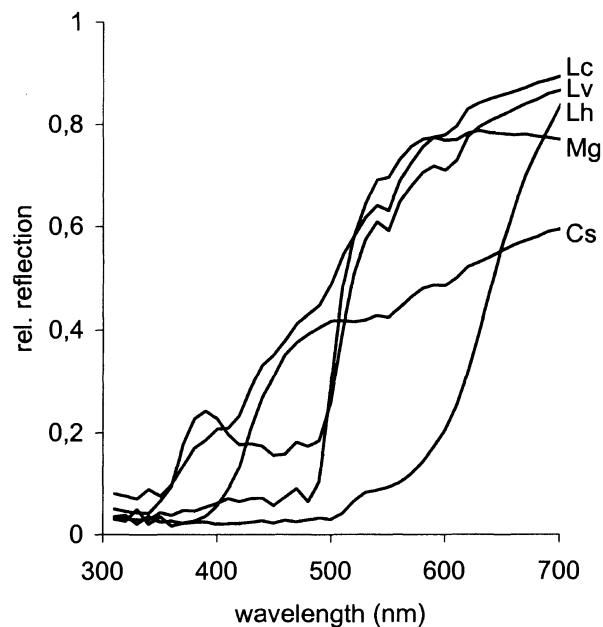


Fig. 9. Spectral reflection properties of pollen. The deep orange pollen of *Lilium henryi* (Liliaceae) reflects mainly light in the long-wavelength range, > 600 nm (*Lh*). *Mimulus guttatus* pollen shows strong absorption at wavelengths < 500 nm, which is typical of many zoophilous flowers (*Mg*). The pollen of *Linaria vulgaris* (Scrophulariaceae) is hidden in the corolla tube and shows a considerable amount of UV-reflection (*Lv*). The white pollen of *Calystegia sepium* (Convolvulaceae) is visible to approaching flower visitors and cryptically coloured against the white corolla (*Cs*). The white *Lotus corniculatus* (Fabaceae) pollen is hidden in the corolla and shows considerable UV-reflection (*Lc*)

relatively large size, as in the Solanaceae (*Solanum*), Gesneriaceae (*Saintpaulia*) (Fig. 6) and Gentianaceae (*Exacum affine*), many of which are buzz-pollinated. The signal action (visual and/or tactile) may be enhanced by an expansion of connectives (*Tradescantia*, Commelinaceae) or the presence of extra signalling structures on the filaments of the stamens, such as hairs in species of *Verbascum* (Scrophulariaceae), Asparagales and Commelinaceae (*Coleotrype madagascarica*, *Tripogandra diuretica*, *Tinnantia fugax*) and nodular thickenings in *Spermannia africana* (Tiliaceae) (Fig. 2) (Osche 1979, 1983a, b; Faden 1992; Vogel 1993; Bernhardt 1996). The secondary increase

in number of stamens, called “dedoublement” (found, e.g. in *Hypericum*, Guttiferae and *Helaleuca*, Myrtaceae) also increases the visual signalling effect of the androecium. The action of the yellow, UV-absorbing coloration of pollen and anthers is improved in almost all animal-pollinated angiosperms by contrastingly coloured corolla petals (Fig. 10).

Some animals consume only the nectar and have no interest in pollen. In some circumstances there can actually be a selection pressure against a conspicuous pollen colour. Bird-pollinated Orchidaeae often have brown pollinia, whereas those of their insect-pollinated relatives are yellow (Dressler 1971). A similar situation was found by Rose and Barthlott (1994) in bird-pollinated and insect-pollinated Cactaceae. In both cases the authors proposed that pollen sticking to a bird’s beak is more likely to induce grooming behaviour if it is conspicuously coloured than if it is approximately the colour of the beak itself.

Supplementation and replacement of the visual signalling function

Although pollen was an immediately available means of attracting flower visitors, there are obvious costs and disadvantages associated with offering it to the visitors as food: pollen is an expensive resource for the plant to produce (Petanidou and Vokou 1990), rich in energy and protein. When enough of it is openly

displayed to attract pollinators, the costs to the plant are increased due to losses; it may be eaten or washed away, the gametes of pollen grains may suffer from exposure to radiation and undergo mutation, and so on. The effectiveness of pollen as an attractant signal depends on the amount made visible, but this is not constant throughout the flowering time of a particular flower. In the course of anthesis it changes in a complicated way owing to the successive breaking open and wilting of the stamens, removal by visiting animals, and other kinds of loss. In monoclinous species, all flowers bear stamens; in diclinous species, only staminate flowers bear stamens and pistillate flowers lack stamens (for exceptions, see below). Pollen-eating flower visitors will restrict their visits to staminate and bisexual flowers and thus pollinate only bisexual flowers. Therefore, bisexuality of flowers is a prerequisite for useful signalling with pollen, but it is also disadvantageous in that it increases the proportion of self- as opposed to cross-pollination. Because of these negative aspects of using pollen as a signal, it is not surprising that in many cases structures have convergently evolved to copy pollen and stamens, serving as signals in their place. The visual signalling action of the androecium can be partially or even completely replaced by that of the imitation stamens (Figs. 6, 7) (Osche 1983b, Vogel 1993, Leins and Erbar 1994). The latter may be differently coloured

Fig. 10. 1st row) Untrained *Eristalis tenax* exhibiting the proboscis reaction towards the test screen of an artificial flower emitting yellow light. *Bombus terrestris* worker encountering a flower for the first time in her life; the antennal response is directed towards the lower lip of *Linaria vulgaris* representing an imitation anther. Pistillate and staminate flower of *Begonia* spec. visited by a honeybee. 2nd row) *Bombus terrestris* worker exhibiting the antennal response at a blue dummy anther of high spectral purity. Colour change of the imitation stamens of *Catalpa bignonioides* (Bignoniaceae) with yellow prechange and dark violet postchange colour. 3rd row) *Iris germanica* (Iridaceae) flower with an imitation androecium mimicking stamens with white filaments and yellow thickened anthers. The normal stamen with its white pollen is situated beneath the petaloid coloured stigma. Hoverfly approaching the stigma of *Crocus sativus* in the pistillate flowering phase. Hoverfly feeding on pollen from stamens of *Crocus sativus* in the staminate flowering phase. 4th row) *Eichhornia crassipes* flower with cryptically coloured pollen and a yellow stamen patch above the androecium. UV-photo of a flower of *Eichhornia crassipes* showing that the UV-absorbing area includes the stamen patch but is not congruent with this area. The sternotribic flower of *Euphrasia roskoviana* presents a yellow stamen patch beneath the androecium



parts of petals, flush with the surface or raised and even developed into three-dimensional structures, and they can present not only visual but also olfactory or tactile signals. The degree to which the signals associated with the stamens are visual cannot be established precisely without experimental studies, because structures added to the filaments and enlarged anthers also enhance olfactory signalling, by increasing the surface area for emission of volatile substances, as well as the stamens' effectiveness as tactile signals.

With other rewards available, the costly pollen (Petanidou and Vokou 1990) can be made less accessible as food. When plants provide nectar as an additional reward, the androecium in many cases is less strikingly coloured and the imitation stamens mark the opening to the nectary, as in *Rhododendron ponticum* (Ericaceae) (Fig. 2) and *Eichhornia crassipes* (Pontederiaceae) (Fig. 10). In many cases the real stamens are entirely invisible to an approaching visitor, being enclosed within the corolla, and imitation stamens completely replace the androecium as signals; examples of papilionaceous flowers include *Colutea arborescens* (Fig. 6), *Tetragonolobus purpureus* (Fabaceae), *Polygala myrtifolia* and *P. vayredae* (Polygalaceae). Here, the two species of each plant family have different types of stamen imitations, i.e. an anther patch on the standard of *C. arborescens*, on the wings of *T. purpureus*, a hairy beard, respectively nodular thickenings of the petaloid wing-shaped sepals of *P. myrtifolia* and *P. vayredae*. It is particularly illuminating to compare related species in which flat patterns resembling stamens mark the entrance to an open perianth tube (e.g. *Euphrasia roskoviana*, *Pinguicula alpina*; Scrophulariaceae) (Fig. 10) with species in which the lower lip formed by an imitation anther in high relief either restricts access (e.g. *Melampyrum pratense*, *Mimulus guttatus*) (Figs. 2, 11) or prevents it altogether (e.g. *Nemesia strumosa*, *Linaria vulgaris*, *Antirrhinum majus*) (Fig. 10), so that the visitor must open the lower lip in order to get at the nectar. In most cases the imitation stamens are situ-



Fig. 11. REM photo of the lower lip of *Melampyrum pratense* with nodular hairs of about the size of pollen grains

ated above the true stamens in cases of sternotribic pollination and below them in cases of nototribic pollination. A signalling function can also be maintained, while reducing the available pollen, by functional subdivision of the androecium into display anthers and viable pollen-producing anthers, as in *Parnassia palustris*, *Verbascum thapsus* (Scrophulariaceae), *Commelina coelestis* (Fig. 7) (Osche 1986) and *Trollius chinensis*. In the kiwi plant *Actinidia deliciosa* (Actinidiaceae) and other bee-pollinated plants, the staminate flowers have normal anthers producing viable pollen while the pistillate ones have anthers with sterile, nutrient-poor feeder pollen (Cane 1993, Goodwin and Steven 1993). In *Parnassia palustris* (Saxifragaceae), *Commelina coelestis* (Commelinaceae) and *Trollius chinensis* (Ranunculaceae) (Pellmyr 1989, 1992b) staminodes without any pollen have evolved.

A separation of pollen production from signalling function can even be achieved within individual stamens. In species of *Salvia* (Lamiaceae) a projection grows out from the stamen so that one theca blocks access to the nectar. It acts as a trigger to activate a rocker mechanism. In *Salvia hians* this theca still

carries pollen, in many other species it is conspicuously coloured. At the end of the rocker is the other theca, which swings down to deposit pollen on the abdomen of the visiting bee. In various species of Melastomataceae different colours distinguish functionally different parts of the anthers, one that releases pollen through a pore and another that acts as a visual attractant (Renner 1989).

Angiosperm flowers are thought to have been originally bisexual and insect-pollinated (Lloyd and Wells 1992), the disadvantages of bisexuality, such as self-pollination, clogging, and need for self-incompatibility, being compensated by the advantages of pollen transfer by insects rather than by the wind. Secondary dichinity reduces self-pollination in monoecious species and prevents it in dioecious ones. In the Cucurbitaceae and in species of *Begonia* (Begoniaceae) the stigmas of the pistillate flowers resemble an androecium (Vogel 1978) (Figs. 6, 10), so that although these flowers offer only nectar, or in some cases neither nectar nor pollen, pollinators are deceived into visiting them by this imitation of the stamens of staminate flowers (Ågren and Schemske 1991, Schemske and Ågren 1995, Schemske et al. 1996). Dukas (1987) observed that honeybees clearly prefer the staminate flowers of *Ecballium elaterium* (Cucurbitaceae), whereas solitary bees visit staminate and pistillate flowers with equal frequency. The oligoleptic solitary bee *Peponapis pruinosa* (Anthophoridae) likewise shows no preference for either staminate or pistillate flowers of *Cucurbita pepo* (Cucurbitaceae) (Willis and Kevan 1995). Dioecy has evolved independently several times in the genus *Solanum* (Solanaceae). In all cases of dioecy in *Solanum*, functionally male flowers have normal anthers, fertile pollen and reduced stigmas while functionally female flowers, which closely resemble male flowers, have stigmas and anthers that appear normal but contain non-functional pollen (Knapp et al. 1998).

Dichogamy can be regarded as a mechanism for reducing self-pollination. The flowers of these species vary in their attractiveness over

the flowering period. Protogynous species do not yet have any pollen with which to reward visitors during the pistillate phase of flowering. In protandrous species the pistillate phase comes last, by which time the stamens may have wilted and no longer contain pollen. Imitation stamens provide these dichogamous species with an attractant signal that is continually effective throughout the flowering period; their presence is often coupled with a reduced signalling function of the actual androecium. Among the protandrous species, *Saintpaulia ionantha* (Gesneriaceae) (Fig. 6) keeps its anthers looking as though they are full of pollen even during the pistillate phase: they do not wilt, remain yellow and have thecae with stiffened walls (Vogel 1978). The protandrous saxifragaceans *Parnassia palustris* and *Saxifraga stellaris* (Fig. 2), like the protogynous scrophulariaceous *Euphrasia roskoviana* (Fig. 10), present a constant signal all during the flowering period in the form of yellow imitation stamens. The dichogamous species of Asteraceae and Euphorbiaceae make the pollen supply more uniform by means of inflorescences, the individual flowers of which are in different phases of flowering at any given time. A remarkable feature of many species of Asteraceae is a subdivision of the inflorescence into peripheral ray flowers and yellow, UV-absorbing central disc flowers, so that the whole thing resembles a large single pollen flower (Classen-Bockhoff 1990, 1996). Furthermore, by means of the subsequent flowering of the disc florets and of the secondary pollen display pollen is produced in distinct portions and hence more uniformly provided throughout the flowering time of the inflorescence (Leins and Erbar 1990, Erbar and Leins 1995). In many *Crocus* species (Iridaceae) the branches of the stigma resemble an abnormally large stamen, exposed so that it can serve as a landing site. Therefore a visitor can deposit the pollen it has brought along onto the stigma before taking on another load of pollen from the lower-lying stamens. The intense yellow colour of the stigmas of the saffron crocus *Crocus sativus* (Fig. 10) is well known: the

stigmas are dried and ground up to make saffron, a yellow dye used in baking cakes.

Another means of reducing self-pollination is for a species to exist as several flower morphs, which vary in the length of the stamen filament (heteranthery) combined with variation in the length of the style (heterostyly). In heteranthetic species 2 or 3 different morphs are distinguished by the varying length of the filaments in the stamens, often in combination with differences in the size and number of the pollen grains (Pacini and Bellani 1986, Dulberger 1992). Because the pollen is exposed to different degrees, a particular flower visitor can often eat or collect pollen more easily from certain morphs than from others. The morphs can be identified visually by the appearance of the stamens, so it is not surprising that flower visitors interested in the pollen show strong preferences for a particular morph, as observed by Wolfe and Barrett (1987) for the dimorphic *Pontederia cordata* (Pontederiaceae). In many trimorphic Pontederiaceae the stamens and pollen are cryptically coloured, whereas in all three morphs the access to the nectar is marked by a yellow, UV-absorbing spot (Fig. 10). This spot, which amounts to an imitation of a stamen, may be absent in self-pollinating populations of the self-compatible *Eichhornia paniculata* (Barrett 1985). In *Lythrum salicaria* (Lythraceae) morph-constant visits cannot result in pollination, as Darwin (1877) showed in artificial-pollination experiments. More recent studies of the flowering biology of *L. salicaria* (O'Neil and Schmitt 1993, Ågren 1996, Ågren and Ericson 1996) do not discuss the striking colour polymorphism of its pollen. That is, anthers and pollen of the short and medium-length stamens are yellow and are displayed in front of the entrance to the nectar-containing perianth tube, whereas the pollen of the long stamens, which project far out of the tube, is green and closely resembles the inconspicuous colour of the green leaves and the pistil; as a result, in all three morphs the access to the nectary is marked by a glowing yellow colour (Lunau 1995) (Fig. 12). Lunau (1996a) proposes that

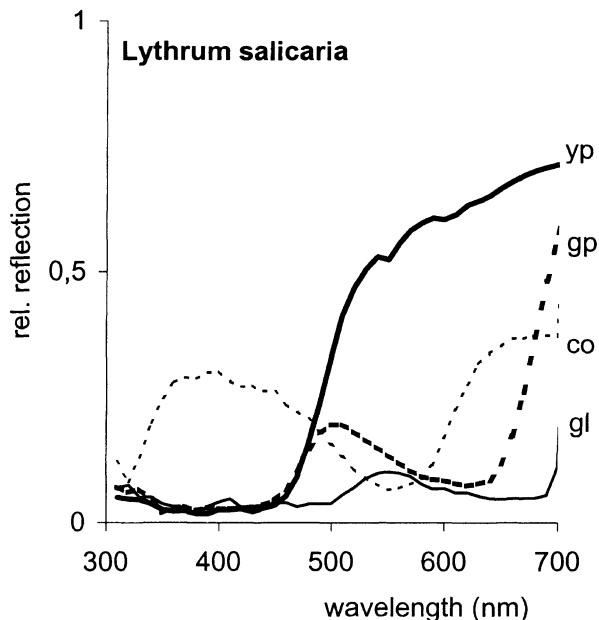


Fig. 12. Spectral reflection properties of *Lythrum salicaria* flowers: green leaves (gl), violet corolla (co), yellow pollen of the stamens with short and medium-length filaments (yp), green pollen of the stamens with long filaments (gp)

this makes it more difficult for the flower visitors to distinguish the morphs from one another. Differences in the coloration of the corollas of dimorphic Primulaceae were noted long ago by Errera (1905). Floral guides that imitate coloured anthers are more often present in the morph of *Primula elatior* and *P. veris* that has long styles and pollen hidden on short stamens in the perianth tube (pin); these markings are situated at the same place in the corolla where the anthers are displayed in the morph with short style (thrum). Again, according to Lunau (1996a), this arrangement might possibly make it harder to tell the morphs apart.

For their protection the stamens are in some cases hidden so far inside the corolla that they cannot act as signals. Very commonly this function is then taken over by imitation stamens. In *Iris germanica* (Iridaceae) the drooping perigonial petal bears a complete beard of hairs that resemble stamens (Fig. 10); at the same place there is a highly arched ridge in *Iris cristata*, only a small rounded ridge in

I. reticulata, an area with a lobed border in *I. japonica* and, finally, a yellow spot in *I. pseudacorus* (Fig. 7). The imitation stamens are all yellow and UV-absorbing. The amount of convergent evolution of imitation stamens in a genus like *Iris* and in other taxa (see above) indicates a relatively recent process on an evolutionary scale (Osche 1983b). Magin et al. (1989) describe the complicated replicas of stamens in *Craterostigma plantagineum* and *Torenia polygonoides* (Scrophulariaceae). In several cases the evolution of imitation stamens in high relief has caused the perianth tube concealing the actual stamens to be closed off; examples include *Linaria* and *Nemesia* (Scrophulariaceae) as well as *Utricularia* (Lentibulariaceae) (Osche 1983b). It is conceivable that changes in the colour of imitation stamens advantageously affect the visitors' behaviour. For instance, in *Aesculus hippocastanum* (Hippocastanaceae), *Polygala chamaebuxus* (Polygalaceae) and *Catalpa bignonioides* (Bignoniaceae) (Fig. 10) the originally yellow imitation stamens become a darker red or violet colour (Lunau 1996b). In the flowering phase during which it is yellow more nectar is offered than in the phase with reddish imitation stamens, and insects are quite capable of learning this association. However, the yellow imitation stamens are also more attractive to inexperienced hoverflies and bumble bees, and this colour is an optimal trigger for innate behaviour patterns (Lunau 1996b).

The deceptive imitation of stamens can go even further: some flowers produce a pseudo-pollen, which is gathered or eaten by their visitors. Cryptic dioecism has been observed in *Rosa setigera* (Rosaceae) and species of *Solanum* (Solanaceae), the pistillate flowers of which produce sterile pollen for consumption by visitors (Kevan et al. 1990, Knapp et al. 1998). The nutrient-poor, sterile pollen of the pistillate kiwi flowers (Goodwin and Steven 1993) evidently serves only to lure pollinators. In Lecythidaceae a single flower produces both food and fertilization pollen, which may be differently coloured (Mori and Orchard 1980). The heterantheric *Lagerstroemia indica* (Ly-

thraceae) produces two kinds of pollen: a group of six large anthers with long filaments produces the real blue pollen, a group of 36–40 stamens with short filaments and small anthers produces the yellow feeding pollen (Pacini and Bellani 1986). A genuine pollen substitute is offered by the *Maxillaria* orchids, which is similar to the pseudopollen of *Polystachya pobequinii* in that it consists of yellow hairs shed by the living cells (van der Pijl and Dodson 1966). *Eria monostachya* produces food hairs from which the heads can be released as pseudopollen (Beck v. Mannagetta und Lerchenau 1914). The extent to which such false pollen are useful as food is unclear. Pollen imitations need not be spherical structures of a diameter such that they are suitable as food or for collecting. The visual and above all tactile properties that mediate the deception can also derive from hairs like those of the anther imitations on the lower-lip petals of *Melampyrum pratense* (Fig. 11), *Mimulus guttatus* (Scrophulariaceae), *Calypso bulbosa* (Boyden 1982) or *Cephalanthera longifolia* (Orchidaceae) (Dafni and Ivri 1981).

The development of pollen signals and neurosensory stimulus-filtering mechanisms matched specifically to pollen signals can be regarded as an example of reciprocal evolution (coevolution) of separate species (Paulus 1988). Flower visitors have evolved neurosensory filter mechanism that allow them to detect visual pollen signals, and flowering plants have increased the intensity of their visual pollen signals so that they are more readily recognized by the visitors. Such evolution has evidently proceeded in two directions: in one the signal comprises complete androecia (e.g. *Begonia*, *Parnassia palustris*, *Digitalis purpurea*, *Iris germanica*) and in the other individual anthers (e.g. *Melampyrum pratense*, *Mimulus guttatus*, *Linaria*, *Colutea arborescens*) (Figs. 2, 6, 10). It is unclear whether this phenomenon is related to different preferences of the various pollinator taxa, to different contexts, such as innate and learned preferences, or to different functions, such as pure visual guide and combined visual and tactile cues.

Conclusions

Pollen is regarded as a means of transporting gametes of seed plants and as a reward for pollinators, but it also serves as a signal in flowers. Various lines of evidence for the visual signalling function of pollen were assembled here. Phylogenetic analysis is concerned with the origin of animal-pollinated flowers and their modification in the course of evolution. Studies of the comparative morphology and physiology of pollen displays are related to the formation of visually discernible colour patterns. Experiments in sensory physiology provide direct demonstrations that visual pollen signals elicit behavioural responses of the animal visitors.

Various features of primarily wind-pollinated plants – the spectral absorption properties of pollen resulting from flavonoid protective pigments, the large amount of pollen produced, the exposure of the pollen by the two thecae of the stamens and the inconspicuousness of other flower organs – make pollen and pollen-producing organs prime candidates to serve as a visual attractant for flower visitors (Osche 1979, 1983a,b). Many of the insects that visit flowers eat the protein- and carbohydrate-containing pollen (Stanley and Linskens 1985, Pacini 1996, Franchi et al. 1996). Among the clues they use to find it are visual, tactile and chemical signals the pollen sends out. Some flower visitors possess innate neurosensory filter mechanisms that respond specifically to visually detectable pollen characteristics. As a result, even inexperienced individuals can orient to flowers in such a way that they make contact with the pollen. It is also possible for visitors to learn to recognize the flowers of their current food plant by visual pollen signals, among other clues.

Beginning with a preadaptive pollen colour, which was yellow and UV-absorbing, in adaptation to pollen-eating flower visitors several pathways are recognised such as: this colour was intensified by additional pigments, especially in the pollenkitt, the corolla enlarged and acquired a contrasting colour, the signalling

function of pollen was transferred to visibly displayed anthers, stamen polymorphism, in which the flower contains both sterile staminodes that are visually striking and inconspicuous fertile stamens, and there was an increase in the number or size of stamens. In many lines of evolution stamens were protected by hiding them in the corolla, and their disappearance was often coupled with the development of imitation stamens that supplemented the signalling action of the androecium and finally replaced it altogether. These imitations ranged from flat spots through raised cushions to completely three-dimensional structures such as staminodes. Imitation stamens have been found chiefly in flowers pollinated by bees and syrphid flies, in the most diverse and widespread geographical regions. It follows that pollen signals are a visual signal component of flowers that is understood by bees and syrphid flies worldwide. Imitation stamens are counterfeit signals, but the degree of the deception varies. They may show the way to pollen of genuine value to the visitor; they may be used by pollen and nectar flowers to give the illusion of more pollen than the flower actually produces; and in the case of orchids that do not even produce nectar and pistillate flowers of nectarless species, they disappoint the visitor completely. The provision of nectar can be accompanied by a reduction or loss of the signalling function of pollen, most commonly in the case of flowers with pollinators that feed predominantly or exclusively on nectar such as butterflies, moths and birds.

The comparative morphological studies of Osche (1979, 1983a,b, 1986), in which the signal character of pollen and stamens and its reproduction in pollen and stamen imitations were worked out, provided an incentive for physiological experiments on the sensory systems of flower visitors which demonstrated the releaser function of these signals. Many field observations have added to our understanding of their significance.

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Pollen viability and longevity: practical, ecological and evolutionary implications

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Abstract. The present article reviews the various definitions and terminology of pollen viability and longevity as well as the various tests of its assessment. We compare the advantages and the disadvantages of each method and suggest some practical implications as revealed by the extensive data. We recognize eight main hypotheses concerning the ecology and the evolution of pollen longevity and critically evaluated them according to the literature. The hypotheses are grouped as follows: (1) Desiccation risk-carbohydrate content; (2) Pollen packaging; (3) Pollen competitive ability; (4) Pollinator activity-stigma receptivity duration; (5) Self-pollination chance; (6) Pollen exposure schedule; (7) Pollen travel distance, and (8) Pollen removal chance.

Key words: Pollen longevity, desiccation, viability, stigma receptivity.

Why study pollen viability?

Assessment of pollen viability is critical for the study of the following aspects of pollination biology: monitoring pollen vigour during storage; genetics and pollen-stigma interaction; crop improvement and breeding programmes; gene bank maintenance; incompatibility and fertility studies; evaluation of pollen germinability after exposure to certain conditions,

and evaluation of dispersal and gene flow (Stanley and Linskens 1974, Heslop-Harrison et al. 1984, Heslop-Harrison 1992, Dafni 1992: 63, Mulugeta et al. 1994, Shivanna and Rangaswamy 1992: 33).

What is pollen viability?

Even a glimpse of the vast literature on pollination ecology and pollen biology shows a wide variety of terms and definitions regarding pollen functional ability. Viability has been defined as "having the capacity to live, grow, germinate or develop" (Lincoln et al. 1982). But viable pollen grains may not actually germinate (in vitro or in vivo) if the conditions are not right. The term viability has also been used to describe pollen grains capable of germinating on the stigma (Morse 1987, Preston 1991, Vaughton and Ramsey 1991, Nissenbaum 1992), germinating in vitro (Shchori, et al. 1992, Beardsell et al. 1993, Lindgren et al. 1995), picking up certain stains (Bernhardt, et al. 1980, Becker and Ewart 1990, Mione and Anderson, 1992, Nyman 1992), and effective seed set following pollination (Smith-Huerta and Vasek 1984).

Some workers have used other terms to better identify what is actually being assessed.

Terms such as stainability (Nybom 1986, Horn and Clark 1992), pollen maturity (King 1960), pollen quality (Knox et al. 1986, Demchik and Day 1996), potential viability (Hauser and Morrison 1964), vigour (Shivanna and Rangaswamy 1992, Snow and Spira 1993), vitality (Vassileva, et al. 1991), germinability (Stanley and Liskens 1974, Pinney and Polito 1990), fertility (Palfi and Koves 1984), relative fertility (Coppens d'Eeckenbrugge and Duval 1993) and fertilization ability (Cruzan 1990) have been favoured at times over the more generally used term viability. Eti et al. (1994) even used the term "semi-viable" and Hoc et al. (1994) used "subviable" to describe lightly stained pollen. Some of these terms have been used in different ways by different workers and sometimes the terms are interchanged within a study (Pinney and Polito 1990, Horn and Clark 1992, McKellar and Quesenberry 1992).

The terms viability, stainability, vigour, germinability, fertility, and fertilization ability indicate different aspects of pollen potential. The terms germinability, fertility, and fertilization ability are more restrictive than viability. A pollen grain may have the capacity to germinate, but not do so due to improper conditions. It may even germinate, but not

have the ability to fertilize an ovule because of some form of incompatibility. These terms better reflect an actual measurement (germination or percentage of fertilization) than does viability. We suggest using different terms for different testing criteria based on the developmental stage of the pollen grain under examination (Table 1). Following the recommendations of Thomson et al. (1994), terms such as viability and vigour should be clearly and operationally defined, or avoided entirely and replaced with more specific terms. All of our suggested terms have been used previously, except stigmatic germinability. We have added that term because conditions differ so much for in vitro and in vivo germination tests, and we suggest that this term will reflect that difference. Pollen longevity will be used here to denote the period in which pollen remains able to germinate on the appropriate (receptive and compatible) stigma.

It is noteworthy that the loss of viability is a continuous variable rather than a dichotomous condition (Thompson in Kearns and Inouye 1993: 33). That implies the need to decide upon an arbitrary threshold to express experimental results. Kumar et al. (1995) defined "pollen viability duration" as the time

Table 1. Terms used to assess the nature of pollen grain viability, their ability to germinate and fertilize ovules

Stage of Development	Criterion	Recommended terminology and citation
Pollen formation	Aborted or infertile pollen grains	Pollen sterility (Shivanna and Johri 1989)
Intact pollen grain	Cytoplasm present	Stainability (Horn and Clark 1992, Coppens d'Eeckenbrugge 1993)
	Plasmalemma intact	Pollen quality (Knox et al. 1986)
	Enzyme activity present	Viability (Lincoln et al. 1982)
		Pollen stainability (Anderson and Ascher 1993)
Germination in vitro	Tube at least as long as grain diameter	Germinability (Stanley and Liskens 1974)
in vivo	Germination on the stigma, tube length	Stigmatic germinability
Fertilization and seed set	Seed production	Fertilization ability (Cruzan 1990)

period in which pollen grains had more than 50% viability. This definition could serve as an experimental yardstick to replace definitions like "pollen longevity in the period in which pollen is viable" (Stanley and Linskens 1974: 56), which is not practical.

Tests for pollen viability

"The most accurate test of pollen viability is the ability of pollen to effect fertilization and seed set" (Smith-Huerta and Vasek 1984, Shivanna and Johri 1989: 90), but it is noteworthy that while seed set certainly indicates pollen viability, the reverse is not true. That is, lack of seed set does not necessarily indicate lack of viability. Methods of determining pollen viability are numerous, and the method of choice depends on the crop (species) and on establishing relationships between the test and the fertility (=the ability to produce seeds) (Hanna and Towill 1995).

All the methods of testing pollen viability depend on many factors (such as enzyme activity, cytoplasm content, plasmalemma integrity and physical conditions of the test such as temperature and exposure to air). None of these methods is able to confirm that a particular sample of pollen is inviable and will not be able to fertilize any plant; they only give a likelihood estimate (Heslop-Harrison 1992).

There are essentially five approaches to pollen viability testing as follows: (1) measures of respiration or chemical conductivity of pollen leachates (rarely used); (2) staining techniques (vital stains for presence of cytoplasm and dyes which indicate enzyme activity); (3) germination (*in vivo* and *in vitro*); (4) content of proline; and (5) capacity to effect seed set (Stanley and Linskens 1974). Because there is no one best test to examine pollen viability *in vitro*, it is recommended that several tests would be used simultaneously to reflect several components of pollen performance (Thomson et al. 1994, Rodriguez-Riano and Dafni 2000). The advantages and the disadvantages of the common methods are summarized in Table 2.

Practical implications

Based on Table 2 we suggest the following four rules of thumb in pollen viability/longevity experiments as follows:

- 1) Record carefully the collecting and storage conditions prior to the test (temperature/RH exposure, desiccation chances, hydration/dehydration status).
- 2) Fresh exposed pollen in the field/greenhouse is preferable to avoid the influence of pre-test uncontrolled factors.
- 3) Test parallel samples of dead pollen (killed by 80 °C, 2 h or in a droplet of ethanol 50%) as a control.
- 4) Test hydrated (1–2 h, 100% RH) vs. unhydrated (dehydrated) pollen.
- 5) Test several procedures simultaneously and then choose the best one for your particular species.

In spite of all the precautions one may consider that pollen judged "dead" in the laboratory may be able to sire seed under natural conditions (Proctor 1998) and vice versa (Table 2).

Because loss of viability is a continuous process in which the activity of many enzymes are involved and are degraded gradually, at different rates, pollen may still have active enzymes even though it has lost the ability to germinate, thus these tests and enzyme activity should be used with caution (Sedgley and Griffin 1989: 271, Sedgley and Hubbard 1993, Georgieva and Kruleva 1994).

Ecological and evolutionary implications

Thomson and Thomson (1992) have already mentioned that pollen viability schedules deserve equal attention to pollen presentation schedules as an object of quantitative study for evolutionary ecologists. The possible factors which should influence pollen longevity are summarized in Fig. 1.

A review of the literature shows that many researchers have already dealt with the ecological and evolutionary implications of pollen longevity and put forward several hypotheses

and ideas that are not necessarily mutually exclusive. We have tried to group them into some broad categories as in relation to: 1. the pollen content structure and packaging; 2. breeding systems and pollen exposure; 3. desiccation; 4. environmental factors and 5. pollinator behaviour and pollination chances. One should consider that this grouping is just for the sake of discussion and the same hypothesis may be included in more than one category.

1. The desiccation risk, carbohydrate content

Pollen under high risk of desiccation will contain sucrose which protects the membrane and confer greater pollen longevity (Hoekstra et al. 1989). Franchi et al. (1996) enlarges this concept stating, "Type of carbohydrate reserves and water content seem to influence the mean life of pollen. Pollen which is not dehydrated at dispersal does not have cytoplasmic polysaccharides and has only a low, or no, sucrose content."

Sucrose seems to stabilize the plasma membranes, keeping them viable during dehydration and transport (Hoekstra and van Roekel 1988, Hoekstra et al. 1989, 1991). The evidence is that sucrose-rich pollen survives for a longer time than sucrose-poor pollen (Hoekstra et al. 1989, Buitink et al. 1996); polysaccharides are also involved in resistance to dehydration (Lisci et al. 1994, Pacini and Viegi 1995, Franchi et al. 1996, Speranza et al. 1997, Nepi and Franchi, this volume).

When pollination occurs rapidly after anther opening, pollen needs little or no protection against desiccation and its reserve may consist mainly of polysaccharides. Conversely, when pollen has a long trip or must wait for a vector, its needs stronger protection against desiccation in time, and starch must be depolymerized to produce sucrose and protect the pollen membranes just before or during pollen maturation (Pacini 1996). Various pathways linking pollen carbohydrates and pollen longevity are summarized in Fig. 2.

2. The pollen packaging – pollen longevity

The larger the number of pollen grains in a package, the higher the protection from desiccation or damaging ultraviolet light (Eisikowitch et al. 1987, Proctor 1998). Pacini and Franchi (1996) recognized several groups of pollen dispersal units as follows: (1) single pollen grains (monads), (2) filiform pollen grains that tangle together, (3) grains adhering together by virtue of viscous substances, (4) grains connected by filaments, and (5) grains joined by common walls. The three types of viscous materials are pollenkitt, tryphine and elastoviscin, all of which function to form clumps with various numbers of grains and enable the dispersal unit to stick to the visitor body (Pacini and Franchi 1996, Hesse and Vogel, this volume).

Tight packaging of several/many pollen grains adhering together creates a defined unit; the larger the number of grains, the lesser the exposure of the individual grain to desiccation. Thus, this aspect may confer greater longevity (see Table 3). It is logical to assume that all these extra materials/structures demand an additional energetic investment.

Vaknin et al. (1999) found in almonds that diluted pollen in talcum on powdered silica-gel caused a reduction in pollen viability. They believed that the separation of the natural pollen clumps into individual pollen grains, caused higher ventilation around individual pollen grains and induced further desiccation and reduced later germinability.

Evidence of individual differences in pollinia longevity in two *Asclepias* spp. indicated 4 days for *A. syriaca* (Eisikowitch et al. 1987) and 1 day for *A. exaltata*. Wyatt and Broyles (1990) suggest that this may reflect habitat differences: *A. syriaca* grows in a drier habitat than *A. exaltata*; the latter's pollinia may be more sensitive to desiccation (Eisikowitch et al. 1987, Proctor 1998). Morse (1987) proposed that the average travel time from pollinia removal to deposition is shorter in *A. exaltata* than in *A. syriaca* and, thus, has less chances for desiccation (see also hypothesis 7).

Table 2. The advantages and disadvantages of the various tests to assess pollen viability

Test	Advantages	Disadvantages
Fruit and seed set measures pollen ability to affect resulting fertilization	*The most authentic and accurate test of viability (Shivanna and Johri 1989: 90)	*Laborious and time consuming (Heslop-Harrison et al. 1984) *More a qualitative than a quantitative test (Shivanna and Johri 1989: 90)
In vivo germination measures the germinability on the proper conspecific stigma	*Simulating natural pollination *Determines SI site(s) *More valid method than in vitro test (Shivanna and Johri 1989: 91)	*Must be accompanied with tests for stigma receptivity (Stone et al. 1995) *Viable pollen may not germinate on the conspecific stigma due to incompatibility factors not related to viability *If seed set is used as a criterion there is a time delay to obtain the results (Stone et al. 1995) *If either seed set or pollen tubes are monitored, it is time consuming and stigmatic surfaces may limit the sample size (Stone et al. 1995) *There is a lack of simple techniques to study pollen tubes in the style (Shivanna and Johri 1985: 91) *Pollen may germinate in the style, but fail to fertilize due to late incompatibility
In vitro germination measures pollen germinability under the specific conditions of the medium and temperature conditions. reveals the state of the reserves, the condition of the membranes and the subsequent rate of reserve conversion (Heslop-Harrison 1979)	*Rapid, reasonably, simple and fully quantitative (Shivanna and Johri 1985: 92) *In many species shows correlation with fruit-set and seed-set (Visser 1955)	*Results can vary enormously depending on species or cultivar, composition of the medium, temperature and duration of the test (Stanley and Linskens 1974: 65–66) *Pollen germination is controlled by the vegetative nucleus and may occur in the absence of a viable generative nucleus (Sedgley and Griffin 1989: 281) *Low germination in vitro may still be fertile and produce fruit set (Johri and Vasil 1961) *Results could be affected by time of pollen collection, storage conditions and pollen density on the culture medium (La Porta and Roselli 1991) *Does not determine SI site(s)
Most commonly used test		*No correlation between pollen germination and fruit set. Negative results may reflect a failure to meet the species specific requirements (Polito and Luza 1988) *Pollen responds differently to a given set of conditions as a function of age and stress (Polito and Luza 1988) *Fresh and desiccated pollen of the same species may need different media for optimal germination (Polito and Luza 1988)

Table 2 (continued)

Test	Advantages	Disadvantages
In vitro germination (continued)		<p>*Very susceptible to environmental conditions, results may depend on the pollen density in the sample (Stanley and Linskens 1974)</p> <p>*Pollen germination rate may be high while pollen tube growth may differ at the same conditions (Gudin et al. 1991)</p> <p>*It is quite common that stored pollen samples that fail to germinate in vitro are capable of producing fruits and seeds (Visser 1955)</p>
Miscellaneous "vital stains" (e.g. methylene blue, neutral red, propionic carmine) show presence of cytoplasm	<p>*Quick and easy, in some cases correlated to in vitro germination or seed set (Nybom 1986, Vassileva et al. 1991, Horn and Clark 1992)</p>	<p>*Tend to stain old and dead pollen (Sarvela 1964, Khatun and Flowers 1996, Heslop-Harrison et al. 1984, Käpylä 1991)</p> <p>*No correlation with germination (Vassileva et al. 1991)</p> <p>*Overestimation of viability in comparison with other methods (Firmage and Dafni, unpubl.)</p> <p>*Pollen grains with cytoplasm are not necessarily fully fertile (Ockendon and Gates 1976)</p> <p>*Many immature or aborted pollen yield positive staining (Stanley and Linskens 1974: 81)</p>
Alexander's stain	<p>*Can distinguish aborted from non-aborted pollen (Alexander 1969)</p>	<p>*Tends to stain old and dead pollen (Heslop-Harrison et al. 1984, Zietsman 1991)</p> <p>*No correlation with germination (Marcellan and Camadro 1996)</p>
Tetrazolium dyes shows the reduction of the colourless salt into a coloured substance in the presence of dehydrogenase	<p>*Accurate results for many taxa (Shivana and Johri 1981: 95–6)</p>	<p>*Results may show no correlation to germination tests (Heslop-Harrison et al. 1984)</p> <p>*Staining may be equivocal (Shivana and Rangaswamy 1992, Parfitt and Ganeshan 1989)</p> <p>*The concentration of tetrazolium salt, temperature and period of incubation needs to be standardized to get optimal results in various pollen samples (Shivanna and Johri 1985: 95)</p> <p>*Tends to overestimate viability (Sedgley and Harbard 1993)</p> <p>*Stain non-viable pollen (Parfitt and Ganesham 1989, Käpylä 1992, Khatum and Flowers 1995)</p> <p>*Need 6 h for staining (Aslam et al. 1965)</p> <p>*Staining disappeared after 10 min (Lansac et al. 1994)</p>

Table 2 (continued)

Test	Advantages	Disadvantages
X-Gal (5-bromo-4-chloro-3-indolyle- β -galactoside) shows activity of galactosidase	*Bright colour, easy to read *Simple test, low variability, high correlation with pollen tube growth <i>in situ</i> (Trognitz 1991)	*Tends to overestimate (Sedgley and Harbard 1993) *Stained dead pollen (Rodriguez-Riano and Dafni, 2000)
Baker's reagent shows alcohol dehydrogenase activity	*Quick and easy test (Jancosa and Webster 1989, Dafni 1992: 67) *Can be adjusted to test for various enzymes *Easy to perform even in field conditions (above 25 °C)	*Stains also dead pollen (Rodriguez-Riano and Dafni 2000) *Gradation of stain in some species (Firmage and Dafni, unpublished)
Benzidine test shows peroxidase activity	*Easy and quick procedure	*No correlation with germination (Heslop-Harrison et al. 1984, Koga et al. 1971, Oberle and Watson 1952) *Staining may be equivocal (Hauser and Morrison 1964) *Medium presents health hazard (Dafni 1992 : 68)
Isatin test examine presence of proline	*Can be used on fixed pollen; quick and easy to perform (Palfi et al. 1988; Palfi and Koves 1984)	*Uneven staining (Käpylä 1991, Firmage and Dafni, unpublished) *Crystals obscure unstained pollen, overestimate viability Suitable only for pollen that contain 1% proline (Palfi et al. 1988)
Sigma peroxidase indicator (No. 390-1) Shows peroxidase activity (p-Phenylenediamine di HCL + catechol)	*Differ clearly between live and dead pollen, results are very distinctive, suitable also for field studies (Rodriguez-Riano and Dafni 2000)	*Sensitive to temperature changes (Rodriguez-Riano pers. comm.) *Sensitive to light, has to be carried out in dark (Rodriguez-Riano pers. comm.)
FCR (fluorescein diacetate reaction) – tests the plasmalemma the intactness of the plasma membrane	*Correlates with germination tests but easier and quicker (Heslop-Harrison and Heslop-Harrison 1970, 1992) Very effective for comparison of parent species vs. F ₁ (Bernhardt and Calder 1981) *Tests two aspects of viability enzymatic activity and an intact cell membrane	*Continuum of colour intensities, the preparation is usable only about 15 min. which in many cases is too short a time for counting (Käpylä 1991, Fritz and Lukaszhevsky 1989) *No correlation with <i>in vitro</i> germination (Vergano et al. 1994, Vallania et al. 1988 Lavi et al. 1996, Sato et al. 1998), and in vivo (Rao et al. 1992) *Must have access to epifluorescence; can't be assessed in field *No correlation with seed production (Vaughton and Ramsey 1991, Rao et al. 1992) *Pollen has to be correctly pre-hydrated (Heslop-Harrison 1992), desiccated pollen may yield low score (Stone et al. 1995)

Table 2 (continued)

Test	Advantages	Disadvantages
FCR (continued)	(Heslop-Harrison and Heslop-Harrison 1970, 1992) *Correlation with germination for a range of species (Shivana and Heslop-Harrison, 1981, Heslop-Harrison et al. 1984) *It is easier and faster than <i>in vitro</i> germination tests	*Needs to be done with the proper osmotic solution for the species being tested (Heslop-Harrison et al. 1984, Nepi and Pacini 1993) *Large variance between tests, large number of repetitions are needed (Trognotz 1991) *Leakage of the produced fluorescein to the test medium may obscure the results (Heslop-Harrison et al. 1984) *May be that desiccated pollen gives low score but gives high one after rehydration (Stone et al. 1995) *Stained dead pollen (Käpylä 1991) *Various degrees of brightness, results are not clear (Khatum and Flowers 1995, Sukhvibul and Considine 1993) *No correlation with seed production (Thompson et al. 1994; Fritz and Lukaszewski 1989) *Because it measures the potential for germination, rather than germination, it may overestimate viability (Shivana and Heslop-Harrison 1981) *No correlation with germination and seed siring (Thomson et al. 1994, Maguire and Sedgley 1997, van der Walt and Littlejohn 1996) *Needs fluorescent microscopy; must be done within time limit; time fluorescence held may be most important element; can show significant variability; time consuming *Desiccated pollen may yield low score (Stone et al. 1995). In some species no correlation with germination (Heslop-Harrison et al. 1984, Singh et al. 1985, Fritz and Lukaszewski 1989, Käpylä 1991, Trognotz 1991, Abdul-Baki 1992, Rao et al. 1992, Nepi and Pacini 1993, Thomson et al. 1994, Khatum and Flowers 1995, Maguire and Sedgley 1997, Lavi et al. 1996, Sato et al. 1998)

3. The pollen competitive ability – pollen longevity

The higher the pollen competitive ability, the lower the pollen longevity (Harder and Wilson 1994). According to these authors there is an

inverse relationship between competitive ability and pollen longevity. This relationship reinforces the impact of time dependent fertilization probabilities in determining the optimal dispensing schedule for a given species,

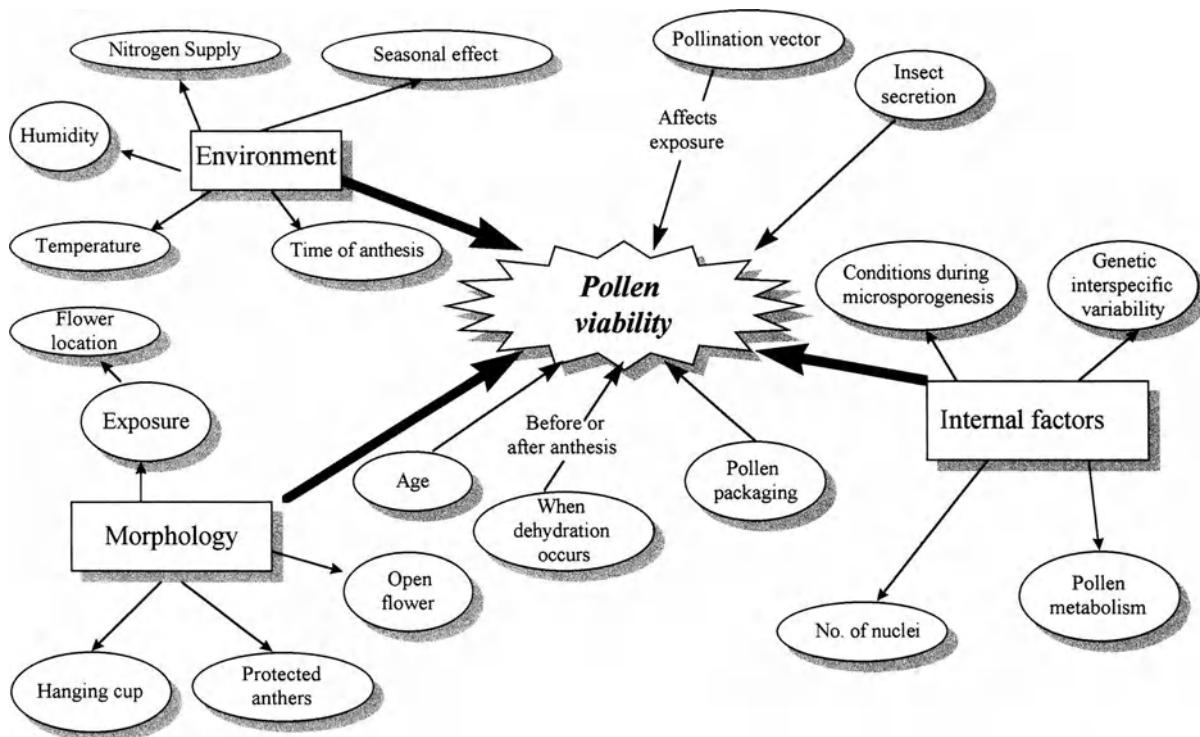


Fig. 1. Factors affecting pollen viability

because losses of pollen viability promote increased early removal in a manner similar to pollen precedence (Harder and Wilson 1994). This tempting hypothesis still awaits experimental corroboration.

4. The stigma receptivity duration – pollen longevity

The lower the pollinator activity, the higher the pollen longevity. It is logical to assume that with low pollinator activity a long exposure of the stigma as well as high pollen longevity may be selected to ensure some rate of pollination. Although seemingly explicit and even trivial, this important aspect is almost completely overlooked. Beardsell et al. (1993) noted that extended periods of stigma receptivity and pollen viability coupled with abundant rewards were selected for early flowering season of the *Thryptomene calycina* (Myrtaceae) in which insect activity is sparse.

Palmer et al. (1989) suggested that high pollen longevity, in conjunction with immedi-

ate and prolonged stigma receptivity, produces conditions highly favourable to the pollen-competition mode of gametophytic selection.

5. The self-pollination chance – pollen longevity

The shorter the pollen longevity, the lower the chances for autogamy. Higher chances for autogamy (e.g. cleistogamy, spontaneous self-pollination and high geitonogamy) should be associated with short-lived pollen whereas exogamy favours relatively long-lived pollen. Supporting evidence is supplied by the observations that conspecific cleistogamous pollen is relatively short-lived in comparison to chasmogamous pollen, as reviewed by Franchi et al. 1996 (see also Lord 1981). Thus, it would be expected that relatively short-lived pollen in clonal self-compatible populations as compared with their non-clonal congeneric species, which are self-incompatible.

In exogamous protandrous species, short pollen longevity prevents self-pollination

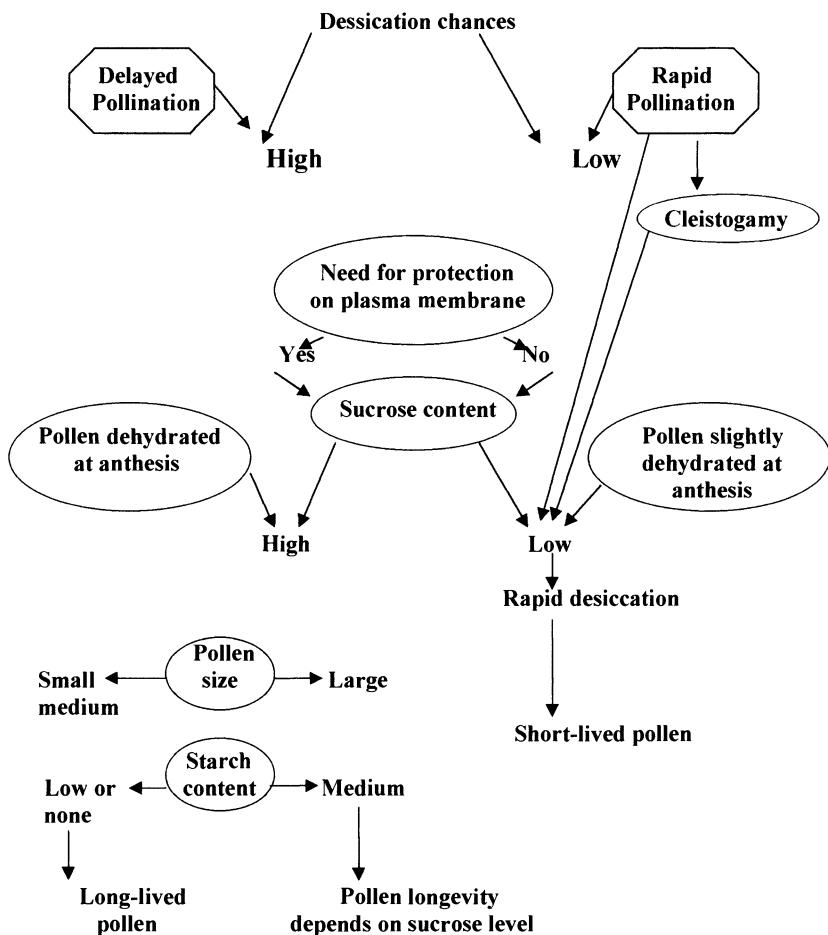


Fig. 2. Pollen carbohydrate content in relation to pollination desiccation and pollen longevity. Sources: Pacini 1996; Franchi et al. 1996; Speranza et al. 1997; Hoekstra et al. 1989, 1991

(Nyman 1992). This author found a correlation between pollen germinability duration and the breeding system in several species of *Campanula*. In exogamous species, optimum pollen germinability is out of phase with the time of stigma receptivity thus preventing self-pollination. On the other hand, in autogamous species, optimum pollen germinability is matched with stigma receptivity.

6. The pollen exposure schedule – pollen longevity

Frequent release of small quantities of pollen is related to short-lived pollen. Short viability of pollen is related to the extended pollen protection (e.g. pollen secondary presentation

schedule (Brantjes 1983). Brantjes (1983) put forward the idea that one might question whether the short pollen viability of Asteraceae is an evolutionary consequence of the extended protection or a selective force for its evolution. Thompson and Barrett (1981) argued that selection on the male function should favour prolonged pollen presentation because that would secure more mating opportunities. Brantjes (1983) invokes weather induced loss of viability as another selective agent with similar effect.

Stephenson and Bertin (1983: 119) argued that male competition will favour a pattern of pollen release that ensures optimum availability to the pollinator. If, for example, pollen viability declines with time, selection

Table 3. Pollen longevity under field greenhouse conditions in relation to pollen packaging, and mode of pollination

Species/Family	Conditions of exposure	Test	Packaging	Longevity	Remark	References
* <i>Oryza sativa</i> (Poaceae)	Open greenhouse	In vivo germination	Monads	4 min	Desiccation reduced viability	Koga et al. 1971
* <i>Triticum aestivum</i> (Poaceae)	Greenhouse	Seed set	Monads	5 min	Desiccation reduced viability	Athwal and Kimber 1970
* <i>Oryza sativa</i> (Poaceae)	Greenhouse	FCR, MTT, Aniline blue, Seed set	Monads	20 min (50%) 60 min	Desiccation reduced viability	Khatum and Flowers 1995, Fritz and Lukaszewski 1989
<i>Fagopyrum esculentum</i> (Polygonaceae)	Room	To complete	Groups	1 h 34–38 h	Desiccation reduced viability	Adhikaire and Campbell 1998
<i>Sorghum bicolor</i> (Poaceae)	Greenhouse	Tetrazolium, seed-set, in vitro germination	Monads	2 h	Desiccation reduced viability	Lansac et al. 1994
* <i>Zea mays</i> (Poaceae)	Greenhouse (Poaceae)	Kernel production	Monads	3 h	Higher RH + lower temp. = longer duration	Jones and Newell 1948
<i>Hermodactylus</i> <i>tuberous</i> (Iridaceae)	Field conditions	FCR, Nitro blue tetrazolium, in vivo germination	Groups	Few hours		Grilli-Caiola and Brandizi 1997
<i>Cucurbita pepo</i> (Cucurbitaceae)	Field conditions	FCR	Groups	24 h	Rapid decline after 6 h	Pacini et al. 1997
* <i>Festuca arundinacea</i> (Poaceae)	Exposed to open air	FCR	Monads	48 h	Rapid decline after 48 h	Pacini et al. 1997
* <i>Mercurialis annua</i> (Euphorbiaceae)	Exposed pollen to open air	FCR	Monads	48 h	Moderate decline	Pacini et al. 1997
<i>Acanthus mollis</i> (Acanthaceae)	Field conditions	FCR	Groups	72 h	Gradual gradient	Pacini et al. 1997
* <i>Chamaerops humilis</i> (Arecaceae)	Field conditions	FCR	Monads?	>72 h	Slow decline	Pacini et al. 1997
<i>Spartium junceum</i> (Fabaceae)	Exposed pollen on a glass slide (open air)	FCR	Groups	>72 h	Gradual decline of viability	Pacini et al. 1997
* <i>Kochia scoparia</i> (Chenopodiaceae)	Greenhouse	Tetrazolium	Monads	2.5 d 1.4 d	High RH + lower temp. = longer longevity	Athwal and Kimber 1970

Table 3 (continued)

Species/Family	Conditions of exposure	Test	Packaging	Longevity	Remark	References
<i>Tectona grandis</i> (Lamiaceae)	Field conditions		Groups	3 d	pollen released at 11 h has the highest viability	Tangmitcharoen and Owens 1997
<i>Asclepias syriaca</i> (Asclepiadaceae)	Field condition	In vitro germination	Pollinia	4 d		Eisikowitch et al. 1987
<i>Cypripedium reginae</i> (Orchidaceae)	Laboratory	In vitro germination	Monads	4 d	Deceptive flower	Proctor 1998
<i>Amianthium muscaetoxicum</i> (Liliaceae)	Field conditions	In vivo germination	Groups	4 d		Palmer et al. 1989
<i>Raphanus sativus</i> (Brassicaceae)	Field conditions	Seed production	Groups	5 d	Stigma receptivity duration 2–3 days	Siddiqui 1983
<i>Platanthera blephariglottis</i> (Orchidaceae)	Greenhouse	Seed production	Pollinia	5 d		Cole and Firmage 1984
<i>Asclepias exaltata</i> (Asclepiadaceae)	Greenhouse	Seed production	Pollinia	5 d		Morse 1987
<i>Pandorea pandorana</i> (Bignoniaceae)	Greenhouse	FCR	Groups	5 d		James and Knox 1993
<i>Blandfordia grandiflora</i> (Liliaceae)	Field conditions	In vivo germination	Groups	> 5 d		Ramsey 1988
<i>Butomus umbellatus</i> (Butomaceae)	Growth chamber	FCR in vitro germination	Groups	6 d		Fernando and Cass 1997
* <i>Picea abies</i> (Pinaceae)	Growth chamber	Germination in vitro	Monads	Several days		Lindgren and Lindgren 1996
* <i>Pinus nigra</i> (Pinaceae)	Growth chamber	Germination in vitro	Monads	Several days		Lindgren and Lindgren 1996
<i>Brassica campestris</i> (Brassicaceae)	Growth chamber	Germination in vivo	Groups	50% viable after 6 d		Katiyar and Gupta 1985
<i>Pogonia ophioglossoides</i> (Orchidaceae)	Laboratory	Germination in vivo	Pollinia	8 d	Deceptive flower	Proctor 1998
<i>Calopogon tuberosus</i> (Orchidaceae)	Laboratory	Seed production	Pollinia	8 d	Deceptive flower	Proctor 1998

		Groups	12 d (33%)	Stigma Vaughton and Ramsey 1991	
<i>Banksia spinulosa</i> var. <i>neoanglia</i> (Proteaceae)	Greenhouse	In vitro germination	Long threads	12 d	Receptivity duration ≈ 10 d Beardsell et al. 1993
<i>Thryptomene calycina</i> (Myrtaceae)	Field conditions	In vitro germination *FCR	Pollinia	37 d	Stigma receptivity duration > 7 d Neiland and Wilcock 1995
<i>Gymnadenia conopsea</i> (Orchidaceae)	Field conditions	In vitro germination + FCR	Pollinia	40 d	Stigma receptivity duration > 7 d Neiland and Wilcock 1995
<i>Dactylorhiza maculata</i> (Orchidaceae)	Field conditions	In vitro germination + FCR	Pollinia	51 d	Stigma receptivity duration, at least 8 d Neiland and Wilcock 1995
<i>Dactylorhiza purpurella</i> (Orchidaceae)	Field conditions	In vitro germination + FCR			

* = Wind pollinated species

might favour the frequent release of small quantities of pollen. This could potentially occur by either sequential anther dehiscence or by the production of numerous open flowers (Lloyd and Yates 1982) or by secondary pollen presentation as noted above. In general, if pollen viability declines rapidly (as a result of internal factors such as rate of metabolism or influence of external factors such as weather), selection may favour pollen presentation in small quantities as in pollen secondary presentation or via many small co-blooming flowers or by sequential anther dehiscence.

7. The pollen travel distance – pollen longevity

The longer the pollen travel distance, the higher the pollen longevity. When the average transit time between anther and stigma, long-lived pollen may be favoured (Proctor 1998). In this line of thought highly dispersed plant species would be expected to have long-lived pollen in comparison with highly dense species. Pacini et al. (1997) found short-lived pollen in two anemophilous species, *Festuca arundinacea* and *Mercurialis annua* (Table 3) and noted that growing crowded together may justify the fairly rapid decline in pollen viability; this close crowding and the consequent short pollen flight means that pollen longevity is unimportant because there is a little selection pressure is exerted on it.

Many orchids are rare and highly dispersed spatially (Neiland and Wilcock 1995) thus pollinia has, frequently, a long distance to travel. These common phenomena are with the same line of the pollinia function as inferred also from the packaging hypothesis.

8. The pollen removal chance

The shorter the pollen removal rate, the shorter the pollen longevity. Pollen is likely to be related to the average time required for pollen to be removed by the floral visitor (Bertin 1988). James and Knox (1993) men-

tioned that where pollinator activity is unreliable or infrequent, pollen longevity may have greater importance in terms of reproductive success. Supporting evidence begins to emerge from studies on two species of *Banksia*. In *B. menziehii* pollen longevity is short (90% uninviabale after 24 h (Ramsey and Vaughton 1991)), but pollen removal efficiency is high (Ramsey 1988). In *B. spinula*, pollen longevity is higher (50% after 8 days) (Vaughton and Ramsey 1991), but pollen removal is incomplete and takes several days (Ramsey 1988).

Many Asteraceae evolved a mechanism to delay the pollen exposure until the pollinator arrives on the flower. The pollen protection ends when the pistil drives out the pollen (Brantjes 1983). Thus, in short-lived animal-borne pollen, a gradual exposure to the environment (as in secondary pollen presentation) will protect the pollen until its removal by the visitors. Asteraceae are known to have short-lived pollen (Hoekstra 1975, Pacini, pers. comm.). We still don't have enough information on the pollen longevity of other families in which pollen secondary presentation has evolved (Yeo 1993).

Interaction of factors

As previously mentioned, flowering characteristics of species or pollination probability may be such that several of the above hypotheses apply to a single species. Beardsell et al. (1993) postulated that long flowering periods, massed small-flower habit, very high pollen production and extended periods of stigma receptivity and pollen viability are all features adapted to unfavourable conditions in which insect activity is rare, and thus the pollination chance is also small. One may extrapolate that pollination syndromes in which flower longevity is extended or has low pollination probability will tend toward a long pollen longevity as well as stigma receptivity duration.

A classic example are the deceptive orchids (note that most of the deceptive flowers, excluding sapromyophily are Orchidaceae (Dafni 1984). The deceptive orchids have low pollination probability systems (Dafni 1984,

Ackermann 1986, Firmage and Cole 1988) that are compensated for by extended flower duration (see also the packaging hypothesis concerning the advantages of pollinia). Additionally, orchids with a nectar reward but pollinator limited such as *Platanthera blephariglottis* have extended flower duration, sequential flowering, tightly packed pollen (pollinia), and extending pollen longevity (Cole and Firmage 1984).

Under harsh conditions in which pollinator activity is low, extended flower duration could compensate for the low-pollination chances (Herrera 1982). A long stigma receptivity duration accompanied by extended pollen longevity may be complementary adaptations to promote pollination. In *Cyclamen persicum*, which flowers from the end of the Summer to the end of the Winter in the Mediterranean, floral longevity is \pm 18–20 days, stigma receptivity is \pm 16–18 days, and pollen longevity is \pm 16–20 days (Swartz-Tzachor 1999).

Bassani et al. (1994) extend this idea also for anemophilous plants in which pollen is dispersed shortly after anthesis and has shorter longevity than in entomophilous species in which pollen is gradually exposed. In general, this hypothesis corroborates the existing data (Table 3). The average longevity (L) for wind-borne pollen is $L_{(10)} = 21.5 \pm 27.2$ h (range 0.05–72 h) whereas for insect-pollinated species, $L_{(21)} = 8.5 \pm 10.4$ d (range 1–40 days).

Concluding remarks and need for further research

A rapid decline of pollen viability may greatly diminish the effective pollen flow and thus may directly influence plant evolution and lead to traits that favour visitation by certain types of pollinators (Roubik 1989: 141). One may consider viscin threads and pollinia as a means for selective pollination by certain agents and as adaptations that also confer greater pollen longevity (see pollen packaging hypothesis).

Environmental factors and especially desiccation risks are considered a main selective force leading to better protection of the pollen

grains. From the evolutionary ecology viewpoint, the possible relation between pollen longevity and pollination chances, pollen competition, and breeding systems is noteworthy. Even if pollen is delivered successfully into the proper receptive stigma, there is no guarantee that it is still viable and one may point out that pollen longevity on the vector body (even at the right location to meet the stigma) may also be a crucial factor in pollination efficiency.

Because there is virtually no "universal test" to assess pollen viability instantly (see Table 2) and because pollen viability loss process is gradual, it is impossible for the researcher to uncover the time-viability decay schedule. Such data are very rare (see Thompson et al. 1994), yet they are crucial for the basic understanding of pollen performance. We still lack a reliable procedure for evaluating pollen viability *in situ* (but see Rodriguez-Riano and Dafni 2000).

Pollen longevity should be studied in relation to the following aspects, with the purpose of establishing its possible adaptive value: (1) intraspecific variability during the season and across contrasting habitats and ecological gradients; (2) interspecific variability among various species, genera and families in relation to pollen content, structure and packaging; (3) in relation to pollen handling and delivery efficiency; (4) as a function of pollen exposure and dispersal schedule, travel duration and distance; and (5) in relation to breeding systems, incompatibility, pollen:ovule ratios, pollen vigour and competition. Most of these aspects have scarcely been investigated in relation to pollen longevity, but are used extensively to explain basic phenomena in plant evolutionary processes.

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The role of electrostatic forces in pollination

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Abstract. This paper reviews research on the role of electrostatic forces in pollination, both in natural and in agricultural systems. Researchers from various fields of biological studies have reported phenomena which they related to electrostatic forces. The theory of electrostatically mediated pollen transfer between insect pollinators and the flowers they visit is described, including recent studies which confirmed that the accumulated charges on airborne honey bees are sufficient for non-contact pollen detachment by electrostatic forces (i.e., electrostatic pollination). The most important morphological features in flower adaptiveness to electrostatic pollination were determined by means of two theoretical models of a flower exposed to an approaching charged cloud of pollen; they are style length and flower opening. Supplementary pollination by using electrostatic techniques is reported, and its possible importance in modern agriculture is discussed.

Key words: Electric charges, electrodeposition, electrostatic pollination, electrostatic powder coating, flower morphology.

Introduction

Electrostatics deals with the electric forces which involve electrons and ions, and with the

related electric fields and potentials. In electrostatic charging, electrons remain relatively stationary and are spread on the surface of the object and concentrated on sharp edges. An object becomes electrostatically charged either by having electrons added to it, thus becoming "negatively" charged or by having them removed from it, thus becoming "positively" charged. Charges of the same sign repel each other and of opposing signs attract each other. The electrostatic force between two charged bodies (F) is described in Coulomb's equation: $F = K(q_1q_2/r^2)$. This force is dependent on the magnitude of the charges (q_1 and q_2), the distance between the charged bodies (r), and on the dielectric coefficient (K). Electrostatic interactions play a major role in a variety of biological processes (Honig and Nicholls 1995), including the pollination of plants, both in nature and in agriculture.

Normally plants possess small negative surface charges under clear fair-day conditions, and are, therefore, surrounded by electric fields of low intensity (Maw 1962). Under unstable weather conditions, as on a cloudy or rainy day, the electric fields can change their polarity and the surface charges become pos-

itive (Warnke 1977). The magnitude of the electric fields depends in part on the chemical composition of the plant, its height and the environment (Erickson and Buchmann 1983). The distribution of the electric field around the plant varies with its shape, and the plant's electrical fields should be greatest near sharp points such as plant terminals including flowers (Dai and Law 1995).

Foraging bees usually possess electrically positive surface charges (Erickson 1975, Yeskov and Sapozhnikov 1976, Warnke 1977, Schwartz 1991, Gan-Mor et al. 1995). When a bee flies through the air it is confronted with electrical currents and its body will be electrostatically charged with "frictional electricity" (Warnke 1977). Warnke (1977) and Thorp (1979) suggested that in the case of pollen-seeking insects, accumulation of pollen on the surfaces of the insects and pollen distribution by the insects are enhanced by the forces of attraction between the insect's positively charged body surface and the generally negatively charged plant with its pollen.

Electrostatic pollination in natural systems

Stanley and Linskens (1974), suggested that while there was no evidence to support the view that electropotential gradients between pollen carried by insects and flower stigmas were involved in pollination, it was possible that long-distance transport of pollen to the flowers was influenced by electrical relationships. Several researchers in various fields of pollination study reported the possible involvement of electrostatics in natural pollination mechanisms.

Wind pollination is associated with the aerodynamics of particle transport and capture (Whitehead 1969, Niklas 1985). It involves airflow patterns which bring conspecific pollen to stigmas of wind pollinated flowers, and is influenced by various factors, including the overall shape of the plant (Niklas and Buchmann 1985, Niklas et al. 1986), the size and shape of the inflorescence (Niklas and Buchmann 1988), and flower morphology and

position on the branch (Niklas and Buchmann 1985). Electrostatic forces have also been shown to influence pollen separation or clumping, which could affect its aerodynamic properties (Erickson and Buchmann 1983). It was later speculated that as pollen grains were carried through the air they acquired a strong positive charge and were thereby electrostatically attracted to the negatively charged female flowers that were concentrated on the terminals of the plants they reached (Erickson and Buchmann 1983). Therefore, together with airflow, electrostatic attraction could form an efficient mechanism for pollen capture by anemophiles (Erickson and Buchmann 1983). However, most reports on the possible involvement of static electricity in wind pollination are speculative, since it is very hard to distinguish between aerodynamic and electrostatic forces in the direction of pollen grains towards the receptive stigma.

Buchmann and Hurley (1978) described the probable role of electrostatics in buzz pollination (vibrational pollination); they considered the attachment of the small, light and dry pollen grains of buzz-pollinated plants (e.g. senna *Cassia* sp., shooting star *Dodecatheon* sp., deadly nightshade *Solanum* sp. and many others) to the body of the pollen-collecting female bee to be largely electrostatic in nature. Pollen deposition on the stigma of these plants is also assumed to be mediated electrostatically (Buchmann 1983, Erickson and Buchmann 1983). However, the actual process of pollen transfer and adhesion to the dry stigmatic surface is as yet unknown.

Eisikowitch (1981) observed that sometimes, under sunny conditions, bumble bees leaving the flowers of oilseed rape (*Brassica napus* L.) created clouds of pollen grains which burst out of the flowers. Some of this pollen was assumed to have reached and adhered to the bumble bee's body, drawn by electrostatic attractive forces (Eisikowitch, personal information). Although wind or spontaneous selfing was considered as the main agent of pollination of oilseed rape, seed yield increased when flowers were subjected to insect pollination.

Corbet et al. (1982) experimented with a living bumble bee (*Bombus* sp.) held down to a cork on a wax block with silken threads. Oilseed rape pollen was sprinkled over the bee and some of the pollen grains that were falling near the bee rapidly drifted towards it and adhered to its body. Electrostatic forces of attraction were thought to be responsible for this phenomenon also.

In avocado (*Persea americana* Mill.), in the pistillate stage, the style is erect and receptive, while the anthers bend up towards the style and dehiscence occurs. Ish-Am and Eisikowitch (1993) found that bees collecting nectar, or nectar and pollen, visited both pistillate and staminate flowers, and, because of the flower structure, they were forced to touch both pistil and anthers. Their visits to flowers in the staminate stage were very short, and they touched the anthers for less than 1 s while hovering, or while landing instantaneously on the flower on the top most position. During these visits, their buzzing sounded lower than usual, and a small cloud of pollen sometimes surrounded them. The possible role of this phenomenon was not discussed, although it is likely that electrostatic forces assisted pollen precipitation (Ish-Am and Eisikowitch, personal information).

Schroeder (1995), stated that electrostatic forces may be associated with the movement of the avocado pollen within a given flower and in the subsequent transfer of pollen grains to other flowers by the honey bee. He conducted simple studies on the effects of electrostatic forces on pollen collection from avocado flowers, and proposed that it is most probable that the pollen adheres to insect bodies not only by the stickiness of the pollenkit but also by the action of electrostatic forces.

Endress (1997) who worked on pollination of *Dillenia* (Dilleniaceae), suggested that the pointed ends of the stylar branches with the stigmas may also be seen as an adaptation to enhance the efficacy of the electrostatic forces involved in the transfer of the dry pollen from anthers to bees and from bees to stigmas.

Theory of electrostatic pollination

The theoretical basis for electrostatic induction suggests that when a charged body is brought into the vicinity of an earthed electrode, a charge of the opposing sign is induced to flow from earth up onto the electrode, to ensure that the electrode remains at ground potential in the presence of the charged body (Law 1975) (Fig. 1A).

Based on this theory, a detailed mechanism of pollination involving electrostatics has been suggested (Hardin 1976, Corbet et al. 1982, Erickson and Buchmann 1983). As an insect carrying an electrical charge approaches a flower, charge of opposite polarity flows into the plant stem and flowers and induces an electric field between the insect and the flower. This electric field grows in strength as the gap between them narrows, and the forces of attraction temporarily created between the airborne insect and the flower initiate the detachment of pollen grains from the anther and their deposition on the body of the insect; the same forces initiate the detachment of the pollen grains from the insect's body and their deposition on various flower parts, including the stigma (Fig. 1B). These processes depend upon physical variables such as the magnitude and spacing of the charge source, the dielectric properties of the media, and the geometry of the flower (Dai and Law 1995). It also depends upon environmental variables such as atmospheric ion concentrations and mobility, and local components of the earth's ambient electric field (Law et al. 1996). Other factors such as flower size, size and hairiness of the bee, level of bee activity and relative humidity are yet to be tested.

The electrostatic force can also function as a short-term sticking factor, especially if the pollen grain is deposited on a dry stigma, enabling it to remain on the receptive surface long enough for proper germination (Woittiez and Willemse 1979).

Chaloner (1986) suggested the possibility that features of exine ornament in pollen of

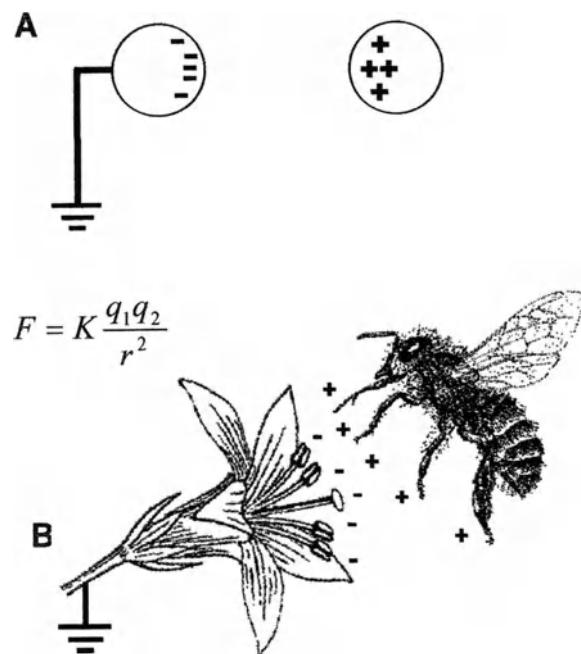


Fig. 1. A scheme of electrostatic induction. (A) A positively charged sphere approaches an earthed sphere and induces an opposite charge; (B) a positively charged bee, carrying pollen grains on its body, approaches an earthed flower and induces an opposite charge, especially on the edges of the flower. In both cases the induction causes temporary forces of attraction between the charged bodies (F). These forces are dependent on the magnitude of the charges (q_1 and q_2) and on the distance between the charged bodies (r). K the dielectric coefficient

entomophilous flowers may have some functional significance in the context of electrostatically assisted pollination. According to this, the exine ornament could function to separate the charged pollen from either the surface of the bee or the flower, to which it is attracted. Accordingly, this would delay charge sharing, and so prolong adherence of pollen to an oppositely charged surface. However, this is pure speculation and requires experimental validation.

Hardin (1976) and Corbet et al. (1982) suggested that when stigmas protrude from the flower and provide the lowest-impedance path to earth, the electric field induced by an approaching charged bee will be the strongest around the stigma. Such a field may enhance

pollen transfer from the bee to the stigma because the stigma will provide the strongest attraction for the charged pollen grains on the body of the insect.

However, on the basis of calculations of relaxation time (the time it takes the charges to flow from the ground through the plant and into the flowers) conducted by Dai and Law (1995) the authors of the present paper do not agree with this statement. Experimental measurements of style and petal conductivity of yellow crooked-necked summer squash ($13\text{ m}\Omega$ (Ohm) and $100\text{ m}\Omega$, respectively) revealed that the relaxation times were 3 ns for the style and 25 ns for the petals (Dai and Law 1995). This difference in relaxation time is so small that it is negligible since the charged body covers the last millimeters in few tenths of a second.

Measurements of the forces involved in electrostatic pollination

Several attempts have been made to measure the forces of attraction induced by the foraging honey bee as it approaches the flower (Corbet et al. 1982, Schwartz 1991, Gan-Mor et al. 1995). In a series of experiments, Corbet et al. (1982) subjected pollen grains of oilseed rape (*Brassica napus* L.) on anthers and honey bees to electrostatic potentials of hundreds of volts as suggested by the measurement of Yes'kov and Sapozhnikov (1976). They showed that a dead honey bee connected to a potential of 750 V (Volt) could cause detachment of oilseed rape pollen from an earthed anther to the bee's body over a distance of 630 μm . Under the same potential, pollen on the bee's body jumped to an earthed stigma over a distance of 376 μm .

In the case of the honey bee, Gan-Mor et al. (1995) were able to show that the average charge on a honey bee after active flight through the air was 23.1 pC (pico Coulomb), with a maximum of 93 pC. The forces required for detaching pollen were $4 \times 10^{-10}\text{ N}$ (Newton), $3 \times 10^{-10}\text{ N}$, and $39 \times 10^{-10}\text{ N}$ for avocado, *Eucalyptus camaldulensis*, and lisianthus (*Eustoma grandiflorum*).

rum), respectively. Mathematical modeling showed that there were cases when the accumulated charge on the honey bee was sufficient for non-contact pollen detachment (Gan-Mor et al. 1995).

Electrostatic pollination and flower morphology

The geometries involved in electrodeposition processes of pollen grains are very complex and difficult to model mathematically. Nevertheless, two mathematical models have been developed in order to describe the complex system of a charged body (insect or pollen cloud) approaching a flower which has a protruding stigma.

A 3-D finite-element model for the analysis of the transient electric field produced by a cloud of charged pollen particles as it approaches and enters a model squash flower was constructed by Dai and Law (1995). The model was coupled with the dynamic space charge and the resulting transient boundary potential on the flower surface. Exactly modeling the charged cloud and its neighboring flower becomes difficult because of the extremely complex geometry of the flower. Therefore, several assumptions were made to simplify the physical model: (1) the cloud's space charge was represented by a uniform space charge density; (2) the charged cloud had a cylindrical shape; (3) the flower consisted of a style surrounded by five segmented cone petals. For instantaneous introduction of a charged pollen cloud, results showed that the electric field immediately above the flower stigma was approximately three times that above the petal edges. The model also showed that the electric field near a flower was affected by the geometry of the flower: as the opening angle between the petals and the style increased, the electric field near the stigma rose while the one near the petals decreased. According to this model, the greater electric fields which prevail when the flower is more open and exposed enable pollen electrodeposition onto the stigma to be maximized.

In another model, the electric field in a system comprising a charged cloud approaching an earthed date (*Phoenix dactylifera* L.) flower was calculated (Bechar 1996, Bechar et al. 1999). A 3-D finite element model was constructed, and a pollen-grain trajectory initiated from the cloud and ending on the flower was simulated. The model flower was simplified by defining its geometry as a 4 mm diameter sphere with a cylindrical pistil, 0.4 mm in diameter attached to its top. It was found that the maximal electric field was concentrated on the pistil top, and it increased as the pistil length increased. The simulation also showed that when electrostatic charging was applied, the pollen density on the pistil top was much higher (up to 225%) than without charging. Two experimental systems were set up, in order to test this model. One experiment with an artificial flower used a 19-mm-diameter steel sphere with a 1.9-mm-diameter, and 19-mm-long rod to represent the style (on a scale of 4.75:1). The artificial flower was exposed to three treatments: It was dusted with uncharged date pollen; with pollen charged by electrostatic corona at 40 kV and 11 mA (for more details see Bright et al. 1978); and with pollen charged by electrostatic corona at 80 kV and 27 mA. It was found that as the voltage and the electrostatic charge density in the pollen cloud increased, the density of pollen on the pistil increased, and the ratio of the pollen density on the pistil to that on the corolla also increased. In another experiment, almond flowers were exposed to the same pollination regimes as those above and similar differences in pollen densities on the stigma were obtained (Fig. 2; Vaknin 1999).

Electrostatic pollination in agricultural systems

Pollination of crop plants is a major factor in achieving sufficient crop set. Crop plants are pollinated by various means: most of them, such as almond (*Amygdalus communis* L.) and apple (*Malus sylvestris* Mill.) by insects (mainly honey bees, *Apis mellifera* L.) (McGregor

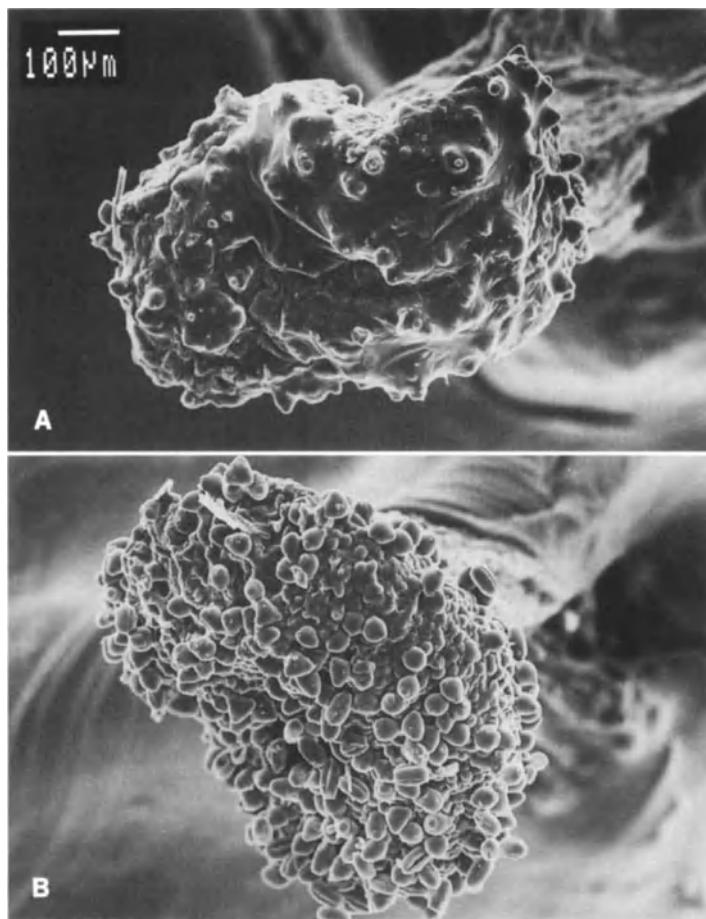


Fig. 2. Pollen grains of almond deposited on the stigmas of almond flowers. (A) The flower was dusted with uncharged pollen; (B) the flower was dusted with pollen charged at 80 kV and 27 mA

1976, Free 1993), others, such as date, olive (*Olea europaea* L.) and pistachio (*Pistacia vera* L) by wind.

In recent years, insufficient pollination has been found to be one of the important causes of low yields in many field and orchard species (Shivanna and Sawhney 1997). Some species require management of pollinating agents, while others benefit from artificial means of pollination, one of which is pollen supplementation (Hopping and Jerram 1980a, b), involving three major steps: (1) pollen collection; (2) pollen storage; (3) pollen deposition on receptive stigmas.

Over the past 35 years, electrostatic forces have been employed in numerous technologies, including the transportation, collection, or separation of materials in the form of powder or small droplets (Bright et al. 1978).

Law et al. (1996), stressed the need for an efficient mechanized pollination method as a solution for pollination deficiencies in modern agriculture. They suggested using the techniques used in electrostatic deposition of powders and liquids, which were available in both industry and agriculture. These techniques generally comprise three distinct operations: (1) the generation and electrification of a charged particulate cloud or stream; (2) the conveyance of these particles to the vicinity of the desired target object; and (3) deposition of the charged particulates on the target (Law 1989).

Law and Cooper (1989) had shown that for plants growing directly in the soil, electrical conduction must be adequate to establish the appropriate distribution of charges to maintain the plant at earth potential during its

interaction with an incoming electrostatic charge. Also, several experiments showed that electrical grounding of plants is adequate for the satisfactory development of electrostatic crop-spraying.

Three widely used particle charging methods are suggested for electrostatic pollen supplementation: (1) electrostatic induction of charge onto a conductive liquid dispersion medium containing suspended pollen grains (Banerjee and Law 1996); (2) triboelectrification (i.e. frictional charging) of pollen grains (Banerjee and Law 1988); and (3) ionized-field charging of the pollen grains by feeding them in an air stream past a high-voltage electrode which incorporates spikes at which corona discharges are formed by the locally intense electric field (Bright et al. 1978). Emerging pollen grains are charged by ion bombardment in the corona region, and are then transported by both aerodynamic and electrostatic forces towards the flowering plant. As the mass of negatively charged pollen grains approaches the targeted plant, they induce positive charging by creating an electron flow inside the plant down into the soil, which keep the earthed plant at zero potential, leaving all the exposed plant surfaces with a temporary positive charge. The resulting electric field forces the negatively charged pollen grains in the cloud front towards the positively charged plant parts.

In the last 20 years, several groups of researchers have tried various techniques for supplementary pollination.

Although experiments on mechanical dusting of flowering apple trees with apple pollen succeeded in increasing fruit set, when the pollen grains were electrostatically charged, pollen deposition in the trees did not increase (Legge 1975, cited by Williams and Legge 1979).

Oltman (1997) described, in a commercial paper, mechanical dusting techniques for various commercially grown plants, including almond, plum (*Prunus* spp.), apple, olive, walnut (*Juglans regia* L.), and pistachio. The machinery consisted of pollen blowers with

discharge nozzles that induced an electrostatic charge on the pollen. Although no scientific experiment was carried out, it seems that farmers in California are now beginning to use this technique regularly, and some even report increases in yield (Vaknin, personal communication).

Electrostatic dusting of larch (*Larix eurolensis* Henry.) was described by Philippe and Baldet (1997). In this experiment, electrostatically charged pollen was blown to the flowering trees by an electrostatic gun and its effectiveness was compared with that of conventional pollen blowing. Results showed that electrostatic dusting deposited three times more pollen on the flowers (15 vs five pollen grains per bract) and enhanced full seed percentage (32 vs 23%) without reduction in pollen viability, although the amount of pollen used was much smaller in the electrostatic procedure.

Bechar et al. (1999) used electrostatic dusting techniques in pollination experiments on dates, and found that by applying electrostatically charged pollen they could raise fruit set by an average of 85 to 175%. SEM analysis of date flowers which were dusted electrostatically or non-electrostatically showed that the stigmas which were dusted electrostatically were totally covered with pollen grains whereas the ones which were dusted non-electrostatically received fewer pollen grains. Similar results were observed with almond flowers (Fig. 2; see also Bechar 1996).

Detailed experiments on several aspects of pollen supplementation in almond and pistachio were conducted by the authors of this paper. They included pollen collection and storage (Vaknin and Eisikowitch 2000) and pollen dilution prior to its application (Vaknin et al. 1999). They had also shown that the use of electrostatic techniques in pollination of almond and pistachio could raise yields by an average of 10 and 20% respectively (Vaknin et al. a, Vaknin et al. b, in preparation). Usually, an increase of 10% or more in fruit set is sufficient to cover all expenses of pollen supplementation and it leaves the growers with

a substantial profit. However, an excess of pollen supplementation could be detrimental as it has been found in walnuts (*Juglans regia* L.; McGranahan et al. 1994). The balance between sufficient and excess pollen supplementation requires further investigation for each crop individually. These studies have just begun to reveal the potential of this technology. More research on this subject will not only lead to improved crops in agriculture, but will also add valuable information on the intricacies of electrostatics in pollination biology.

Summary

The possible involvement of electrostatic phenomena in pollination processes in nature has been a subject of discussion and speculation for the last 20 years. The theory of the electrostatic aspect of pollination describing the effect of a charged bee approaching a flower, has been widely known for many years, but only recently has its occurrence in nature been partially confirmed. It was shown that the accumulated charge on airborne honey bees is sufficient to achieve non-contact pollen detachment by electrostatic forces. Two mathematical models were constructed to describe the complex system of a charged body such as a honey bee approaching a flower. The models predicted that the electric field near a flower and its stigma should depend mostly on the geometry of the flower. The electrical field above the flower stigma was predicted to be much greater than those above the petal edges. Also, as flower opening and style length increased, the electrical field above the stigma increased and that above the petal edges decreased. Harnessing these electrostatic forces by using them as the basis of a method of pollen supplementation in agriculture could prove to be a major breakthrough in modern management of pollination processes. However, much more research is required, to elucidate the relevant phenomena, in both natural and agricultural systems.

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Pollen grains: why so many?

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Abstract. My objective is the examination of selective forces that affect pollen number. Relationships among other floral traits of animal-pollinated plants, including pollen size, stigma area and depth, and the pollen-bearing area of the pollinator may affect pollen number and also provide a model to examine how change in one trait may elicit change in other traits. The model provides a conceptual framework for appreciating intra- and inter-specific differences in these traits. An equivalent model is presented for wind-pollinated plants. For these plants the distance between putative mates may be the most important factor affecting pollen number. I briefly consider how many pollen grains must reach a stigma to assure fruit set. I use pollen-ovule ratios (P/Os) to examine how breeding system, sexual system, pollen vector, and dispersal unit influence pollen grain number. I also compare the P/Os of plants with primary and secondary pollen presentation and those that provide only pollen as a reward with those that provide nectar as part or all of the reward. There is a substantial decrease in P/O from xenogamy to facultative xenogamy to autogamy. Relative to homoeious species the P/Os of species with most other sexual systems are higher. This suggests that there is a cost associated with changes in sexual system. The P/Os of wind-pollinated plants are substantially higher than those of animal-pollinated plants, and the available data suggest there is little difference in the pollination efficiency of the various animal vectors. The P/Os of plants whose pollen is dispersed in tetrads, polyads, or pollinia are substantially lower than those of species whose

pollen is dispersed as monads. There was no difference in the P/Os of plants with primary and secondary pollen presentation. The P/Os of plants that provide only pollen as a reward were higher than those that provide nectar as a reward. All of these conclusions merit additional testing as they are based on samples that are relatively small and/or systematically biased.

Key words: Animal-pollination, breeding systems, duration stigma receptivity, pollen grain number, pollen grain size, pollen packaging units, pollen-ovule ratios, secondary pollen presentation, sexual systems, stigma area and depth, wind-pollination.

In a series of papers my colleagues and I proposed that pollen-ovule ratios (P/Os) reflected pollination efficiency (= total source efficiency of Inouye et al. 1994), i.e. the likelihood of a pollen grain reaching a stigma. That conclusion was based on the differences in P/Os among species with different breeding systems (Cruden 1977, Cruden and Lyon 1989) and differences between species whose pollen is dispersed as monads vis-à-vis those whose pollen is dispersed in polyads or pollinia (Cruden 1977, Cruden 1997, also see Cruden and Jensen 1979). Subsequently, we showed that stigma area relative to the pollen bearing area of the pollinator was negatively correlated with P/O (Cruden and Miller-Ward 1981). In essence, we provided functional explanations for the variation we observed in floral traits and

how interactions among those traits influenced their evolution (also see Cruden 1997).

Alternative hypotheses were proposed to explain a number of the relationships we had observed. For example, the negative relationship between pollen grain number and size was explained as a trade-off between number and size (e.g. Charnov 1982). Local mate competition was invoked to explain the lower P/Os of autogamous and facultatively xenogamous species relative to xenogamous species (Charnov 1982). Both local mate competition (e.g. Queller 1984) and the reduction of sibling rivalry (Kress 1981, Uma Shaanker 1988) were proposed as explanations for the quite low P/Os of plants whose pollen is dispersed in polyads and pollinia. Because substantive data sets are inconsistent with the predictions of most of these hypotheses (Cruden 1997), and little additional data, either pro or con, has become available, I deal only briefly with the relative merits of the model developed below and alternative hypotheses that address particular relationships or traits.

To understand pollen grain number one has to understand the ecological milieu in which pollen grain number evolves. Thus, my initial objective is the examination of floral

traits that contribute to male function and how they might interact evolutionarily. In addition to pollen grain number, these include pollen grain size, stigma depth and area, and the pollen-bearing area of the pollinator. I discuss relationships among these traits in animal-pollinated flowers and then discuss an equivalent set of relationships in wind-pollinated flowers. I consider how many pollen grains per ovule are necessary for fruit and seed set. I examine other traits and interactions that may affect the evolution of pollen number, hence pollen-ovule ratios (P/Os), including breeding system, sexual system, pollen vector, and packaging of pollen grains. I also consider ecological factors that affect pollen grain number and quality.

Animal-pollination: relationships among floral traits

A number of floral traits involved in animal-pollination may interact evolutionarily (Fig. 1). These relationships provide a model for examining how evolutionary change in one trait might effect change in another (see below, Cruden 1997). The model is based on several assumptions. First, seed number, hence ovule number,

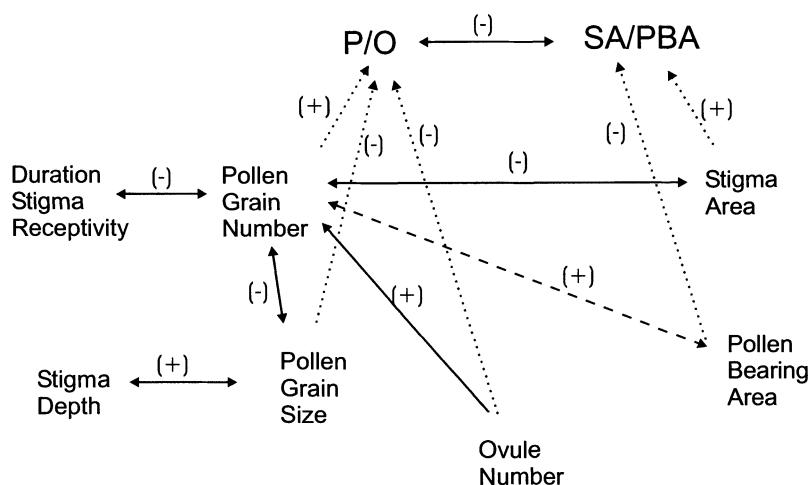


Fig. 1. Relationships among floral traits in animal-pollinated plants. The solid lines indicate relationships that were demonstrated empirically (see text). The dashed line indicates a proposed relationship. The dotted lines indicate that the pollen-ovule ratio (P/O) or stigma area (SA)/pollen bearing area of the pollinator (PBA) might be influenced by change in a given trait

responds to selective pressures that affect offspring survivorship. Thus, in a species that occurs in a variety of habitats seed number and size, hence ovule number, might differ substantially among populations. There is no evidence that ovule number responds to evolutionary change in other floral traits. Second, in most species floral traits respond to selection independently of one another (see Cruden 1997). Third, pollinator numbers and/or their dependability may vary among habitats and pollen number and/or other traits reflect those differences (e.g. Cruden 1976, Ramsey 1993). Fourth, selective pressures that affect ovule number are rarely correlated with those that affect pollinator numbers and/or their dependability.

Differences between populations in ovule and pollen number, hence P/Os, in *Blandfordia grandiflora* R. Br. and *Epacris microphylla* R. Br. in eastern Australia are consistent with the assumptions. Tableland plants of *B. grandiflora* produced more ovules (169.2 vs. 123.5) and nearly twice as many (96.4 vs. 48.7) but smaller seeds (2.13 vs. 3.14 mg) than coastal plants. Tableland plants produced substantially fewer pollen grains per flower (930,000 vs. 1,410,000) and had lower P/Os (5600 vs. 11,500) (Ramsey 1993, also see Ramsey et al. 1994). Stigma pollen loads were equivalent in the Tableland and coastal populations (288 vs. 272) (Ramsey 1993), thus, the Tableland plants were more efficiently pollinated. Because the differences in reproductive traits were retained in a transplant garden they were ecotypically adapted, i.e. the various traits, including pollen grain and ovule number, were adapted to local or regional environmental conditions. Ovule and pollen number also differed between populations of *Epacris microphylla* (Cruden and Lyon pers. obser.). The flowers of a population in the Blue Mountains produced larger and fewer ovules compared to those of a tableland population (35 vs. 120) and fewer pollen grains per flower (1000 vs. 1600). The fewer and larger ovules suggest fewer and larger seeds. The P/O of the Blue Mountains population was twice that of the tableland population

(140:1 vs. 70:1), which suggests differences in pollinator efficiency.

Pollen grain number and ovule number. In general, and other things equal, pollen grain number has to be positively related to ovule number. This relationship was observed across families (Cruden and Miller-Ward 1981) and within families (Kirk 1993, López et al. 1999, Plitman and Levin 1990, Small 1988).

Variation in pollen grain and/or ovule number may occur within individuals and/or within or among populations. For any given individual pollen grain and/or ovule number may be more or less constant, e.g. in *Linaria* (Arnold 1982a, also see Devlin 1989), or change during the growing season (see below). In homoecious species variation in pollen grain number may reflect differences in pollen grains per anther and/or anther number, e.g. Bosch (1992), and, in species with other sexual systems, the percentage of male flowers per plant and/or male plants, e.g. in *Lignocarpa* (Webb 1984). Likewise, ovule number may vary as a function of the number of ovules per carpel or ovary, carpels per flower, female flowers per plant and/or female plants.

In homoecious species, i.e. those with only hermaphroditic flowers (see Cruden and Lloyd 1995), both pollen and ovule number may vary within a plant during the flowering season. This need not involve a change in the P/O. In *Clarkia unquiculata* Lindl. both pollen grain and ovule number decreased during the flowering season and the P/O did not change (Vasek et al. 1987). In *Raphanus sativus* L. (Young and Stanton 1990a) and *Chamaecrista fasciculata* Michx. (Frazee and Marquis 1994) both pollen grain number and ovule number decreased across the flowering season. Likewise, in other species ovule number decreased through the flowering season (e.g. Arnold 1982b; Olesen and Warncke 1992; Ornduff 1986; Pellmyr 1985, 1987; Vogler et al. 1999). In contrast, in *Diervilla lonicera* Mill. ovule number increased during the flowering season and coincided with increased pollinator activity (Thomson 1985). In these cases pollen production was not determined.

Some of the seasonal variation in pollen grain and ovule number may reflect dichogamy. In populations of protandrous plants or those with protandrous flowers the pollen from the first flowers to open will not reach a stigma and the plants suffer a loss in reproductive success through male function. Brunet and Charlesworth (1995) suggested that this might result in plants investing less in male function in first opening flowers and relatively more in female function. Thus, one might expect to see an increase in male function and a decrease in female function during the flowering season. Protogyny should produce the opposite pattern. There is mixed support for the hypothesis (Brunet and Charlesworth 1995). The data from *Clarkia* (see above) is inconsistent with the model and that from *Aralia hispida* (see below) is consistent with the model. The hypothesis merits testing because only the first flowers to open on the first plants to flower suffer a reproductive loss, and it is not intuitively clear that this loss, which may have no genetic basis, is sufficient to select for lower pollen production in first opening flowers.

Within plant variation may be independent of season. In *Muntingia* the number of anthers increased from flower to flower within a fascicle and the last opening flowers rarely set fruit (Bawa and Webb 1983). In essence, the first flower of a fascicle was primarily female in function and the last opening primarily male in function.

Pollen grain number and ovule number may vary among plants within a population (e.g. Devlin 1989). Two patterns of variation occurred among individuals of the species that my colleagues and I studied (see Cruden 1977, Cruden and Miller-Ward 1981, Cruden and Lyon 1989). In most species there was no relationship between pollen grain number and ovule number and in a few species there was a positive relationship between them (also see Mione and Anderson 1992). The positive relationship is consistent with the observation that large flowers may contain more pollen grains and ovules than small flowers (e.g. Small 1988, López et al. 1999, also see Dudash

1991). In both *Raphanus sativus* and *Polemonium viscosum* Nutt. pollen grain number, but not ovule number, was positively associated with flower size (Stanton and Preston 1988, Galen and Stanton 1989, also see Mione and Anderson 1992). In no species was there a significant negative relationship between pollen grain number and ovule number.

Pollen grain number and/or ovule number may vary among populations of a species. Such variation may be relatively small and no one has demonstrated a selective basis for it (e.g. Affre et al. 1995, Cruden 1977, Díaz Lifante 1996, Ramsey et al. 1994, Vuille 1988). Such variation may simply reflect sampling error, or have an environmental basis (see below). In some species there was substantial variation among populations that reflected differences in breeding system (e.g. Affre et al. 1995, Dahl 1989, Thomas and Murray 1981, also see below). Finally, there were substantial differences in P/Os among populations with the same breeding system that may reflect pollination efficiency or another selective force. For example, in two populations of *Corokia cotoneaster* Raoul quite different P/Os (19,762:1 and 13,634:1) were associated with pollinator guilds composed primarily of flies and bees, respectively (Webb 1994). Fruit sets of 16% and 24% in the two populations were consistent with the bees being more efficient pollinators.

Variation in pollen grain number also occurs in species with other sexual systems. In andromonoecious species pollen grain number may vary as a function of the number of pollen grains per anther and/or the percentage of male flowers a plant produces. In *Aralia hispida* Vent. the number of hermaphroditic flowers decreased with umbel order and that of male flowers increased (Thomson and Barrett 1981), and the number of pollen grains per flower and pollen grain size increased with umbel order (Thomson et al. 1989). As a consequence the P/O increased substantially with umbel order. Likewise, in *Cimicifuga americana* Michx. the percentage of male flowers increased during the flowering season (Pellmyr 1986). Parallels with

the variation in pollen grain number per flower and seasonal change among homoecious plants is found in andromonoecious *Leptospermum scoparium* J. R. et G. Forst. In this species the percentage of male flowers varied among plants and within plants they tended to open first (Primack 1980a, b), and pollen grain number presumably decreased during the flowering season. Finally, in *Caesalpinia pulcherrima*, an andromonoecious legume, the percentage of male flowers varied among populations and was negatively correlated with the number of pollinator visits (Cruden 1976, Cruden and Hermann-Parker 1979).

Pollen grain number and size. A negative relationship between pollen grain number and size is well documented (e.g. Cruden 1997, Small 1988, Vonhof and Harder 1995, also see Cruden and Miller-Ward 1981, Dulberger and Ornduff 1980, Mione and Anderson 1992, but see Knudsen and Olesen 1993, López et al. 1999) and has been discussed by numerous authors. Many attributed the relationship to a simple trade-off between number and size, (e.g. Charnov 1982, Vonhof and Harder 1995) and much of the data is consistent with such an interpretation. However, a number of selective pressures may influence pollen grain size and/or number, including stigma depth and stigma area (see below). The small openings in the anthers of vibratory flowers may select for small pollen grains, e.g. in *Solanum* and various *Cassia*. Larger pollen grains might clog the openings. In other species with porate anthers the anthers dehisce in response to bees tripping a trigger hair, e.g. in various Ericaceae. Here also small pollen grains may be adaptive. Likewise, in *Ficus* selection might favor small to tiny pollen grains. First, small grains are less likely to be removed from the body when a wasp enters a syconium. Second, small pollen grains would probably be favored in those figs whose wasps store pollen in pockets in the exoskeleton because the wasps have to carry sufficient numbers of pollen grains to pollinate all the flowers in a syconium.

Pollen grain size and stigma depth. The positive relationship between pollen grain size

and stigma depth, i.e. the distance from the surface of the stigma to the transmission tissue in the style, reflects the distance a pollen tube has to grow to reach resources in the style. This relationship is based on two sets of observations. First, in a number of taxa there was a positive relationship between pollen grain size and stigma depth (Cruden and Lyon 1985, Williams and Rouse 1990, also see Small 1988) and in several of them there was no relationship with style length (Cruden and Lyon 1985). Second, there is now a considerable body of literature on the types of resources in the style or transmission tissue, which do not occur in the stigma, that are utilized by growing pollen tubes. For example, in Kiwi-fruit (González et al. 1996) and some Apiaceae and Brassicaceae (Cruden and Lyon 1985) starch is stored in the style and as the pollen tubes grow through the transmission tissue the starch grains disappear. The underlying assumption is that larger pollen grains contain more nutrients than smaller ones, at least those that store starch, and this allows their pollen tubes to grow through deep stigmas that can not be traversed by pollen tubes from small pollen grains.

Pollen number and duration of stigma receptivity. The likelihood of a stigma receiving sufficient numbers of pollen grains is increased by the length of time it is receptive. Differences in the period of receptivity may reflect ecological and/or physiological factors or genetic differences among populations. In many species unpollinated stigmas remained receptive longer than pollinated stigmas (e.g. Devlin and Stephenson 1984, Richardson and Stephenson 1989). In *Nemophila menziesii* H. and A. this allowed efficient fly pollination in habitats where the bees that usually pollinated the flowers were absent (Cruden 1972).

Inter- and intra-specific differences in duration of stigma receptivity are associated with different traits that affect the likelihood of pollination (see Dafni and Firmage this volume and discussion of *Potamogeton* below under wind-pollination). In *Blandfordia grandis* the stigmas of Tableland plants remained

receptive longer than those of coastal plants and this was associated with lower P/Os and more efficient pollination (Ramsey 1993). In gynodioecious *Sidalcea oregana* flowers of female plants received fewer visits and stigma pollen loads increased slowly relative to flowers on hermaphroditic plants, but the female flowers stayed open longer and eventually their stigma pollen loads were equivalent to those of hermaphroditic flowers (Ashman and Stanton 1991). Also, if pollen grains remain on the vector for a substantial period of time, as those of *Caesalpinia pulcherrima* do (Cruden and Hermann-Parker 1979), the likelihood of a pollen grain reaching a stigma increases.

Pollen grain number and stigma area.

Because stigmas with a large area contact more of the pollen-bearing area (PBA) compared to stigmas with a small area fewer pollen grains should be required for equivalent pollination success. In both *Sympionema* and *Isopogon* (Proteaceae) pollen grain number and stigma area were negatively related (Cruden 1997). This relationship is not easily accommodated in models that examine male and female function as essentially separate, sometimes antagonistic, properties.

Pollen grain number and pollen-bearing area. Pollen number should be positively related to the pollen-bearing area of the pollinator (PBA) (Cruden 1997). If the PBA decreases the pollen grains are closer together thus increasing the likelihood of a pollen grain contacting a stigma and pollen number should decrease. Conversely if the PBA is large the pollen grains are farther apart and it takes more pollen grains to achieve a density that is equivalent to species with smaller PBAs.

Pollen grain number and size, stigma area and P/O. The interactions described above (see Fig. 1) provide a means for predicting the direction of change in a trait in response to change in a second trait. For example, a decrease in pollen grain number should be associated with an increase in pollen grain size, an increase in stigma area, and/or a decrease in the pollen-bearing area. The complimentary

differences between the morphs of distylous species nicely illustrate some of the predictions. Legitimate pollinations involve the larger numbers of smaller pollen grains in long-styled plants and the smaller SAs of the short-styled plants and smaller numbers of larger pollen grains in short-styled plants with the larger SAs of long-styled plants. In contrast to most distylous species *Amsinckia grandiflora* and some populations of *Hedyotis caerulea* the floral morphs produced equivalent numbers of pollen grains but long-styled plants produced smaller pollen grains (Ornduff 1976, 1980). Thus, it is reasonable to assume that pollen grain number in long styled plants had decreased. The decrease in pollen number was associated with an increase in stigma area in the short styled plants. Data from *Cryptantha* (Casper 1983) provide a similar picture (Cruden 1997).

Comparisons within and between families are consistent with the relationships observed in distylous species. In Fabaceae most species in *Medicago* had larger SAs, lower P/Os, and produced fewer and larger pollen grains than species in *Trigonella* (Small 1988). Likewise, large pollen grains, low P/Os (< 1000:1), and large SAs are characteristic of species in Cactaceae, Cucurbitaceae, Onagraceae, and Nyctaginaceae (Cruden pers. obser., Nassar et al. 1997, Nepi and Pacini 1993, Osborn et al. 1988). In contrast, species in *Amsinckia* (Ornduff 1976), *Cryptantha* (Casper 1983), *Cassia* (Dulberger 1981), and *Isopogon* (Cruden 1997) had small SAs, tiny pollen grains and high P/Os (> 25,000:1).

Stigma area/pollen-bearing area and P/O.

Stigma area relative to the pollen-bearing area directly influences the likelihood of a pollen grain reaching a stigma, hence pollen grain number and P/O. At one extreme are species with quite low P/Os and SAs that are large relative to the PBAs. For example, in *Isotria verticillata* (Willd.) Raf. the PBA was a small area on the backs of bees and the P/O was 3.9 (Mehrhoff 1983). Based on the illustrations provided, the SA was approximately the same size or somewhat smaller than the PBA. Likewise, in *Cabomba caroliniana* Gray the

SA was approximately the size of the PBA (see photos in Schneider and Jeter 1982) and the P/O was 62 (Osborn et al. 1991). In *Stylium* (Stylidiaceae) pollen was placed with great precision on a relatively small area on the pollinator (1–2 mm² in Western Australian species) (Armbruster et al. 1994), and the stigmas were large relative to the PBA. The P/Os of two eastern Australian species were ca. 55:1 and 102:1 (Cruden pers. obser.). In contrast, the stigmas of *Cassia* spp. (Dulberger 1981) and *Isopogon anethifolius* (Salisb.) Knight (Cruden pers. obser.) were situated within a tiny cavity at the tip of the style, which was small relative to the PBA, and the P/Os were high.

Finally, the relationships discussed above (see Fig. 1) provide a mechanism for understanding intra- and inter-specific differences in the various traits, e.g. in distylous species. Such differences may reflect different responses to a common selective pressure. For example, an increase in ovule number might elicit an increase in pollen number, or, secondarily, an increase in pollen size and/or an increase in stigma area. When the differences are large and easily detected the adaptive significance can be deduced (e.g. Ramsey 1993). If the response involves small changes in several

traits, the differences among populations in any one trait may be subtle and the evolutionary significance difficult, if not impossible, to discern.

Wind-pollination: relationships among floral traits

A different set of relationships exists among floral traits of wind-pollinated plants (Fig. 2). The primary relationship is that between pollen number and the distance between putative mates. Because pollen grains are lost from the vector due to gravity and become more dispersed as they are moved away from their origin, pollen grain number, hence P/Os should be positively related to the distance between putative mates. It follows that seed set will decrease with distance from a pollen source (e.g. Allison 1990, Berry and Calvo 1989, Honig et al. 1992) and be positively correlated with pollen number (Allison 1990). As in animal-pollinated plants there may be a negative relationship between pollen grain number and both SA, e.g. in *Taxus* (Weis and Hermanutz 1993), and the duration of stigma receptivity. Also, the higher the point of release the longer pollen grains remain in the air, thus pollen grain number should reflect the height of release. In monoecious plants male

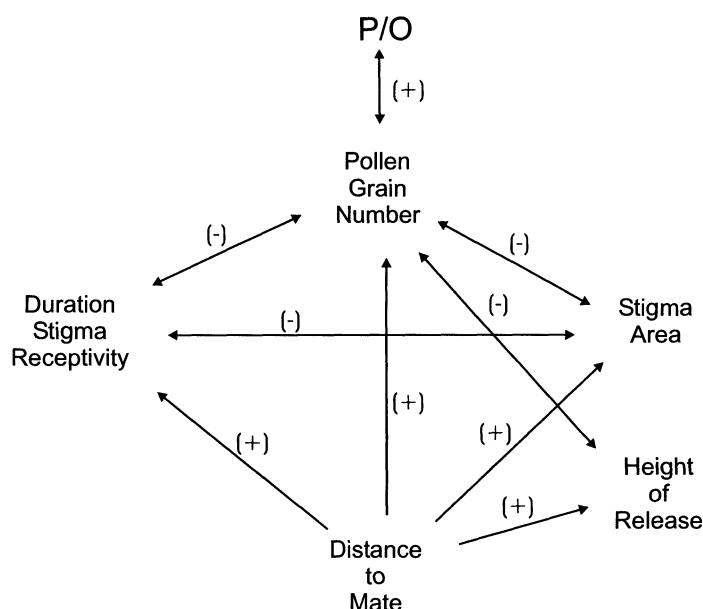


Fig. 2. How various relationships among floral traits and distance between putative mates might affect pollen grain number and the pollen-ovule ratio in wind-pollinated plants. It is likely that all of the traits interact evolutionarily

flowers should be borne higher on the plant or inflorescence, e.g. in *Zea* and *Myriophyllum*. In protogynous homoeocious and monoecious species the inflorescence or petiole of individual flowers might elongate following the female phase. It follows that there are at least four evolutionary responses that might result from insufficient pollen grains reaching the stigmas of a population, i.e. an increase in pollen number, stigma area, duration of stigma receptivity, and/or height of pollen release (Fig. 2).

The interaction between inflorescences and/or flowers and the wind creates an aerodynamic environment in which pollen grains can contact and adhere to a stigma (Niklas 1985). There are considerable differences in inflorescence architecture, e.g. among Cyperaceae, Poaceae, and Potamogetonaceae, as well as considerable differences among stigmas, e.g. exserted and feathery in Poaceae and Cyperaceae and sessile in Potamogetonaceae. There are no data as to whether pollination efficiency, hence pollen number, varies as a function of inflorescence architecture and/or floral morphology.

Several relationships that are important in animal-pollinated plants probably have minimal selective impact in wind-pollinated plants. There is probably no relationship between pollen grain number and size and minimal differences in stigma depth because of the constraints on pollen grain size. Although ovule number must dictate the minimal number of pollen grains produced, the distance between

putative mates appears to be the most important factor in determining pollen grain number.

Preliminary data from temporally dioecious *Potamogeton* (Table 1) are consistent with the model. Most of the species can be placed into one of three categories based on flower number per inflorescence, how long the stigmas were receptive, and the distance between male and female phase inflorescences. The differences among the three groups were striking. The inflorescences of species with 2–8 flowers per inflorescence were the closest together, had relatively small SAs, stigmas that were receptive 1–2 days, and the lowest P/Os. Those with 10–20 flowers per inflorescence were intermediate in all respects. The inflorescences of species with 20–30 flowers were the farthest apart, had the largest SAs, stigmas that were receptive for 4–7 days, and the highest P/Os. Further, the height of pollen release increased from group to group (Cruden pers. obser.).

The exception to the above pattern is *Potamogeton zosteriformis* Fern. (Table 1) and it demonstrates that natural selection may produce quite different combinations of pollen number, stigma area, and duration of stigma receptivity. The inflorescences of this species were the farthest apart of all the species I studied and the stigmas the largest. However, the stigmas were receptive for only 2–4 days. Compared to other species with intermediate sized inflorescences, i.e. *P. gramineus* L. and *P. richardsonii* (Benn.) Rydb., the large SA and

Table 1. Traits of species in *Potamogeton* that may affect pollen-ovule ratios. Flower number per inflorescence, stigma area (mm^2), duration of the female phase (in days), the distance between putative mates, and the P/O are given

	Flower Number	Stigma Area	Female Phase	Distance between	P/O
<i>P. foliosus</i>	4–8	small	1–2	short	9000:1
<i>P. pusillus</i>	2–6	small	1–2	short	9406:1
<i>P. filiformis</i>		0.38		short	2446:1
<i>P. richardsonii</i>	12–16	intermediate	2–4	intermediate	25000:1 ^a
<i>P. gramineus</i>	14–20	0.76	2–4	intermediate	
<i>P. zosteriformis</i>	14–20	3.11	2–4	long	32000–39000:1 ^a
<i>P. nodosus</i>	24–32	1.12	4–7	long	26500:1 ^a
<i>P. natans</i>	24–30	1.50	4–5	long	36000:1 ^a

^a Pollen-ovule ratio from Philbrick and Anderson (1987)

high P/O of *P. zosteriformis* may reflect the greater distances between putative mates. Further, relative to species with equivalent P/Os, i.e. *P. natans* L. and *P. nodosus* Poir., the larger stigma of *P. zosteriformis* may compensate for the shorter duration of stigma receptivity.

Pollen number and fecundity: how many does it take?

The numbers of pollen grains reaching the stigmas has to be sufficient to account for the fertilization of the available egg cells (not ovules!), produce a pollen population effect (see below), provide some minimal level of sexual selection, and swamp the effect of incompatible or low quality pollen. A limited amount of data suggests that some minimal amount of pollen must be available to pollen vectors or reproductive success decreases. Horovitz and Beiles (1980) reported that seed set declined in gynodioecious *Hirschfeldia incana* (L.) Lagreze-Fossat when the number of female plants in garden populations exceeded that in wild populations. In monoecious *Calyptrogyne ghiesbreghtiana* Linden ex. H. Wendl. fruit set was higher when male phase plants outnumbered female phase plants (Cunningham 1995). In this species the number of male phase plants usually exceeded that of female phase plants because the male phase lasts 4–5 nights and the female phase usually lasts two nights. However, in gynodioecious *Saxifraga granulata* L. flowers on female plants set fewer seeds, which is consistent with pollen limitation, but the survivorship of seedlings to reproductive adults was greater from those seeds (Stevens 1988). In this instance pollen limitation was not detrimental even though fecundity was reduced.

Stigma pollen loads must be sufficiently high to produce a pollen population effect, i.e. a sufficient number of pollen grains per ovule to trigger germination and/or produce viable pollen tubes (e.g. Schemske and Fenster 1983, also see Cruzan 1986). This number may be smaller in species with large pollen grains and larger in species with small pollen grains. Further, in

some species stigma pollen loads have to be sufficiently large to account for some minimal number of developing seeds. For example, in *Ruellia bourgaei* Hemsl. and *Bartsia alpina* L. a minimum of 10 seeds have to develop to assure fruit development. Fruits with fewer developing seeds aborted (Cruden pers. obser., Molau 1991, also see Hannan and Prucher 1996, Schemske and Fenster 1983, Stephenson 1981).

Because larger and/or mixed parent stigma pollen loads frequently result in higher quality offspring (e.g. Niesenbaum 1999), it seems reasonable that plants will produce sufficient pollen grains to produce some minimal level of sexual selection. Thus, pollen grain number should be large enough to account for stigma pollen loads that exceed the number of ovules in the ovary and sufficient pollen carry-over to produce multipaternal stigma pollen loads (e.g. Campbell 1998). However, pollen production can not be so high that the pollen produced by one plant swamps the stigmas of the next plant visited by a pollinator. This would reduce or eliminate pollen carry-over and limit considerably the number of potential mates. In essence, both minimal and excessive pollen production may have a detrimental effect on fitness.

The available data suggest that a minimum 4–6 pollen grains per ovule are necessary for maximum seed set (e.g. Murcia 1990; Schuster et al. 1993; Snow 1982, 1986). The data represent animal-pollinated species with low P/Os, e.g. *Mirabilis jalapa* L. (Cruden 1977), and high P/Os, e.g. *Cassia* (Dulberger 1981), wind-pollinated species, e.g. dioecious *Staberoha banksii* Pillans (Honig et al. 1992), and self-pollinating plants, e.g. the cleistogamous flowers of *Viola nephrophylla* Greene (Cruden 1977). Stigmatic pollen loads of this magnitude should be sufficient to produce a pollen population effect, greatly reduce the likelihood of a low quality pollen tube reaching an ovule, and insure some sexual selection.

Pollen-ovule ratios and pollination efficiency

In the following sections I use P/Os to examine hypotheses concerning traits and/or relation-

ships that may affect pollen number. First, I briefly review the relationship between P/Os and breeding system. Next, I consider P/Os in the context of sexual systems and pollen vectors. I also examine the P/Os of plants whose pollen is dispersed in tetrads, polyads, and pollinia because earlier work suggested they were lower than those of species whose pollen is dispersed as monads (Cruden 1977, 1997). Finally, I examine two additional hypotheses: first, the P/Os of species with secondary pollen presentation are lower than those of species with primary pollen presentation (see Yeo 1993), and second, species that offer only pollen as a reward produce more pollen per ovule than those that provide nectar as a reward (e.g. Vogel 1978; Pellmyr 1985, 1986).

My conclusions should be viewed as hypotheses that require testing. With the exception of the breeding system data, the sample sizes are small and may include species from just one or a small number of families. For example, all of the gynodioecious species are in Apiaceae and all the gynomonoecious species are in Asteraceae. Although comparisons across families are relatively compelling, comparisons within genera and families may be equally instructive. The data were analyzed with a Mann-Whitney *U*-test or Wilcoxon two-sample test (Sokal and Rohlf 1969).

Pollen ovule ratios and breeding systems

There is a strong correlation between P/O and breeding system (Fig. 3). P/Os decreased from xenogamous to facultatively xenogamous to autogamous species ($X > FX: t = 9.825; n = 310,86; p << 0.001$ and $FX > A: t = 6.218; n = 86,77; p << 0.001$). The relationship holds within species (Affre et al. 1995, Dahl 1989, and Hannen and Prucher 1996), genera (Feliner 1991, Lawrence 1985, Mione and Anderson 1992, Sharma et al. 1992, Spira 1980, Vuille 1987), and families (Feliner 1991, Lawrence 1985, Plitmann and Levin 1990, Preston 1986, Schlising et al. 1980, Short 1981, Vasek et al. 1987, Webb 1984). However, there is tremendous variation in P/Os among species

with equivalent breeding systems (Fig. 3). The P/Os of most xenogamous, animal-pollinated species are between 1200:1 and 8,000:1. But in some families, e.g. Onagraceae, the P/Os of most xenogamous species are less than 500:1 and those of species with other breeding systems are correspondingly lower (Cruden pers. obser., Vasek and Weng 1988). In other families, e.g. Boraginaceae, the P/Os of xenogamous species are extremely high (30,000:1–200,000:1) and the P/Os of species with other breeding systems are frequently higher than average (see Cruden 1977, Cruden and Lyon 1989).

The differences in P/Os between xenogamous, animal-pollinated plants and related species with other breeding systems also characterize wind-pollinated species and their facultatively xenogamous and autogamous relatives (Cruden 1977, Hammer 1978, Sharma et al. 1992). In addition to having higher P/Os, the stigmas of xenogamous *Plantago* were receptive 4–5 days prior to the dehiscence of the anthers whereas the autogamous species were homogamous (Sharma et al. 1992). The little available data indicate that facultatively xenogamous and autogamous species derived from wind-pollinated species have higher P/O's than species with equivalent breeding systems derived from animal-pollinated, xenogamous species (e.g. Cruden 1977, Sharma et al. 1992).

For many species the P/O is sufficient to correctly identify the breeding system but for xenogamous species with relatively low P/Os the P/O may confuse rather than clarify. Many xenogamous species with low P/Os have large pollen grains and/or large stigma areas (see above). Thus, both the P/O and pollen grain size and/or other traits should be used to determine a plant's breeding system in lieu of determining it experimentally (see Cruden and Lyon 1989).

Pollen-ovule ratios and sexual systems

The available data suggest that homoecious species have lower P/Os than those with other sexual systems (Fig. 4) and that the P/Os of

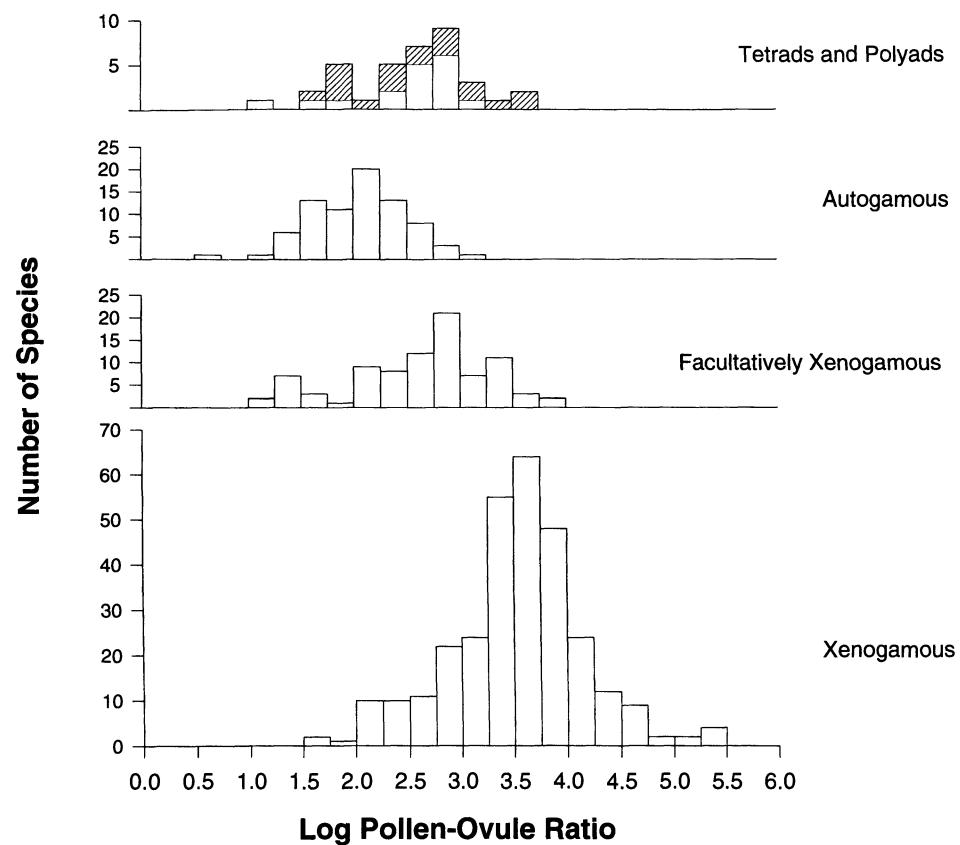


Fig. 3. Distribution of pollen-ovule ratios of animal-pollinated, homoecious species and those of species with other breeding systems. The differences between breeding systems are significantly different (see text). Tetrads are indicated by open bars and polyads by hatched bars. The P/Os of species with tetrads and polyads were equivalent (see text)

wind-pollinated species are higher than those of animal-pollinated species with the same sexual system (Fig. 4).

Dioecy. In animal-pollinated plants the P/Os of dioecious species (median = 10,655:1, quartiles = 4050:1, 67,500:1) were higher than those of homoecious species (median = 3450:1, quartiles = 1550:1, 7338:1) ($t = 3.308$, $n = 19,310$, $p < 0.001$) (see Webb 1984; Mione and Anderson 1992). Likewise, the P/Os of the two wind-pollinated dioecious species (Lisci et al. 1995, Ramirez and Seres 1994) were higher than those of most wind-pollinated homoecious species (Fig. 4; $t = 4.531$, $n = 2,22$, $p < 0.001$). Also, the P/Os of the two wind-pollinated, dioecious species were higher than those of animal-pollinated dioecious species ($U = 35$, $n = 2,19$, $p < 0.025$).

Gynodioecy. The only P/Os for gynodioecious species are in animal-pollinated Apiaceae and they are higher than those of related homoecious species (Webb 1984). The increase in P/Os in most species was a result of greater pollen production per flower and/or increased numbers of male flowers. In a number of gynodioecious species seed production in hermaphroditic plants was lower than in female plants (e.g. Ågren and Willson 1991, Maki 1993), which may reflect the resources needed to support increased pollen production (but see Ågren and Willson 1991).

Monoeagy. Across families the difference between the P/Os of animal-pollinated homoecious (see above) and monoecious (median = 10,194:1, quartiles = 1479:1, 18,310:1) taxa was not statistically significant ($t = 1.735$,

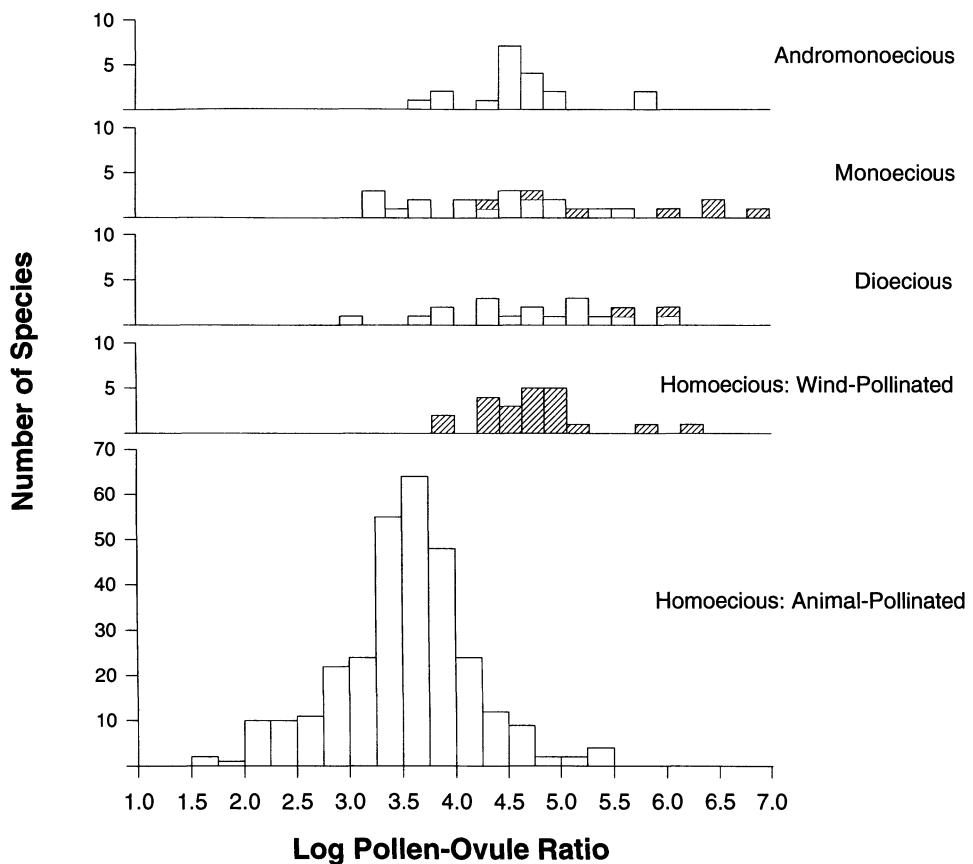


Fig. 4. Distributions of pollen-ovule ratios of animal-pollinated (open bars) and wind-pollinated (hatched bars) species with different sexual systems. The P/Os of wind-pollinated homoecious species are higher than those of animal-pollinated species. Likewise, the wind-pollinated dioecious and monoecious species have higher P/Os than animal-pollinated species with the same sexual system. Animal-pollinated dioecious and andromonoecious have higher P/Os than animal-pollinated homoecious species. The P/Os of animal-pollinated homoecious and monoecious species are equivalent

$n = 19,310$, $p > 0.05$). In contrast, the P/Os of the monoecious, wind-pollinated species were significantly higher than those of homoecious wind-pollinated species ($t = 2.347$; $n = 7,22$; $p < 0.02$). This is probably attributable, at least in part, to the distance between individuals as 7 of the 8 monoecious species were trees, which are generally further apart than herbaceous plants. Also, the two homoecious wind-pollinated species with the highest P/Os were woody plants. Finally, the P/Os of wind-pollinated species were higher than those of animal-pollinated species ($U = 110$, $n = 18,7$, $p < 0.005$). Also, in *Espeletia* (Asteraceae) the wind-pollinated species produced substantially more

pollen per disk floret and many more disk florets per head relative to the animal-pollinated species (Berry and Calvo 1989).

Andromonoecy. Across families the P/Os of andromonoecious, animal-pollinated species (median = 14,983:1, quartiles = 9936:1, 26,020:1) were higher than those of homoeocious species (median = 3450:1, quartiles = 1550:1, 7338:1) ($t = 4.111$, $n = 20,310$, $p < 0.001$). Approximately half of the andromonoecious species were in Apiaceae and the same pattern was observed (Lindsey 1982, Schlesmann 1982, Webb 1984). Likewise, in Rhamnaceae the P/Os of andromonoecious taxa were higher than those of homoecious

taxa (Cruden 1977; Medan 1993, 1994; Medan and D'Ambrogio 1998; Oliveira and Sazima 1990).

Gynomonoecy. In gynomonoecious species the pollen produced by the hermaphroditic flowers was thought to be sufficient to account for the pollination of small numbers of female flowers (Horovitz and Beiles 1980, also see Lloyd 1979). However, in *Senecio*, the P/Os of 17 gynomonoecious taxa were, on average, 25% higher than those of 7 homoecious, xenogamous taxa (Lawrence 1985) ($U = 92$; $df = 17,7$; $P < 0.025$). The only other gynomonoecious species whose P/O was known also was in Asteraceae. In these gynomonoecious taxa the hermaphroditic disk flowers greatly exceeded the number of pistillate ray flowers, a situation in which one might expect little or no increase in pollen production.

The selective reasons for high P/Os in animal-pollinated, dioecious species is not clear and more data are needed from both animal- and wind-pollinated monoecious species to know whether their P/Os are higher or equivalent to those of homoecious species. The higher P/Os of gynomonoecious and gynodioecious species (Lawrence 1985, Webb 1984) may reflect selection to maintain the pollen pool available to vectors above some critical level. As the frequency of female flowers or plants increases, pollen grain number has to increase, or another trait has to change to maintain minimal stigma pollen loads, or fecundity and/or offspring quality will decrease (see above).

Finally, the data discussed above suggest that there may be a cost associated with the evolution of alternative sexual systems and that may help to explain their rarity.

Pollen vectors

Wind-pollination. Wind-pollinated, xenogamous plants produce large numbers of pollen grains (see Ackermann this volume, Pohl 1937, Proctor and Yeo 1972), and because most flowers have just a single ovule their P/Os should be high relative to those of animal-pollinated

plants. The P/Os of the few wind-pollinated, homoecious plants whose P/Os have been determined (Fig. 4; from Campbell 1982, Dajoz and Sandmeier 1997, Hammer 1978, Osborn et al. 1991, Pohl 1937, Sharma et al. 1992, Tomlinson et al. 1979) were higher than those of homoecious, animal-pollinated plants (wind: median = 22,150:1, quartiles = 9,238:1 and 36,000:1; animal: median = 3,450:1; quartiles = 1,550:1 and 7,338:1) ($t = 5.406$; $n = 22,310$; $p << 0.001$). Finally, even though half of the wind-pollinated species for which there were data are in *Potamogeton* ($n = 11$) (Cruden pers. obser.; Philbrick and Anderson 1987), and there were little or no data from such families as Poaceae, Cyperaceae, and Restionaceae, it seems reasonable to conclude that homoecious wind-pollinated plants have higher P/Os than those of animal-pollinated plants.

The comparisons among wind-pollinated species with different sexual systems were based on quite small sample sizes. However, there is reason to believe that the observed relationships are correct. In dioecious and many monoecious species the distance between putative mates is probably greater than in homoecious species. In dioecious species if the sexes are equitably dispersed half of a plant's neighbors will be of the same sex. Second, if the dispersion of plants has an ecological bias, e.g. female plants in moister sites and male plants in drier sites (e.g. Freeman et al. 1976), there may be a substantial distance between male and female plants. Based on distances between putative mates monoecious species should not produce more pollen than related homoecious species, because nearest neighbors will not be farther apart. However, many monoecious species are temporally dioecious and the dispersion of male and female phase plants will be equivalent to those of dioecious species. Hence, their P/Os should be higher than those of related homoecious species.

Animal-pollination. Based on the available data it appears that various insect groups and birds are equally efficient as pollinators. Most of the homoecious species whose P/Os and

pollinators were known were bee-pollinated, and there were few data from species pollinated by insects in other orders. Based on that data the P/Os of bee- and bird-pollinated species were equivalent ($t = 0.862$, $n = 103,19$, $p > 0.4$) as were those of bee- and fly-pollinated species ($t = 0.832$, $n = 103,8$, $p > 0.4$). The P/Os of 6 bat-pollinated species were low relative to bee-pollinated species ($t = 2.391$, $n = 103,6$, $p < 0.02$) but five of the six were in Cactaceae (Nassar et al. 1997) and their P/Os did not differ from three bee-pollinated cacti ($U = 8$, $n = 5,3$, $p > 0.1$). Cacti have low P/Os regardless of pollen vector. There were too few P/Os from species with other vectors to justify an analysis. Species in Polemoniaceae (Plitman and Levin 1990) were not included in the analyses because information on their pollinators was not available.

That different animal vectors may be equally efficient in transferring pollen is consistent with the observation that a variety of vectors were pollinators of plants whose pollen is dispersed in tetrads, polyads and pollinia (Hesse et al. this volume; Johnson and Edwards this volume). Tetrads were dispersed by bees, birds, flies and beetles (Cruden and Lyon pers. obser., Lloyd and Wells 1992, Norman et al. 1992, Olivera et al. 1990) and polyads were effectively transferred by bats, bees, birds, hawkmoths, and settling moths (Bernhardt et al. 1984; Cruden 1977, 1997; Hernández 1989; Hopkins 1984; Koptur 1984). Massulae of various orchids were dispersed by bees, flies, and lepidoptera (Johnson and Edwards this volume, Neiland and Wilcock 1995). Likewise, pollinia of orchids and milkweeds were transferred by a variety of animals.

The relative effectiveness of different vectors merits additional study. However, the examination of related taxa may not prove any more revealing than comparisons of unrelated taxa. Bees were more efficient pollinators of *Corokia cotoneaster* than flies (see above), but among xenogamous Onagraceae fly-pollinated species ($n = 5$) had lower P/Os than those pollinated by bees ($n = 17$), hummingbirds

($n = 5$), hawkmoths ($n = 8$) and settling moths ($n = 5$) (Cruden pers. obser.). In Polemoniaceae flowers pollinated by butterflies had higher P/Os than flowers pollinated by bees, hummingbirds, and flies (Plitmann and Levin 1990). Finally, based on a limited survey of a diverse group of tropical monocots Ramirez and Seres (1994) observed that the P/Os of beetle and/or fly flowers were higher than those of flowers pollinated by bees, bats, and birds.

Tetrads, polyads, and pollina

In general, the P/Os of species whose pollen grains are dispersed in tetrads and polyads (see Cruden 1977, 1997; Oliveira and Sazima 1990; Jacquemart 1997; Kenrick and Knox 1982; Knudsen and Olesen 1993; Koptur 1984; Lloyd and Wells 1992; Norman and Clayton 1986) were lower than those of species whose pollen was dispersed as monads (Fig. 3; $t = 7.577$, $n = 37,310$, $p \ll 0.001$). There was no difference between the P/Os of species whose pollen was dispersed in tetrads vs. polyads ($t = 0.122$; $n = 20,17$; $p > 0.9$). The general pattern was mirrored in several families. For example, in Epacridaceae the P/Os of *Dracophyllum secundum* R. Br. (372:1), *Epacris purpurascens* R. Br. (87:1), *E. reclinata* Cunn. ex Benth. (139:1), and *Rupicola apiculata* (Cunn.) Telford (219:1) were lower than those of *Brachyloma daphnoides* (Smith) Benth. (456:1), *Leucopogon parviflorus* (Andrews) Lindley (648:1), and *Styphelia triflora* Andrews (1072:1), whose pollen grains were dispersed as monads (Cruden and Lyon pers. obser., also see Knudsen and Olesen 1993).

A similar pattern is found in species whose pollen is dispersed in polyads. In *Xyris*, the P/O of *X. juncea* R. Br. (15:1) was lower than those of *X. operculata* Labill. (125:1) and *X. gracilis* R. Br. (142:1), whose pollen was dispersed as monads (Cruden 1997). In Pyrolaceae the P/O of *Chimaphila umbellata* (L.) W. Barton was less than half that of *Orthila secunda* (L.) House, whose pollen was dispersed in monads (Knudsen and Olesen 1993).

The pollen of very few families is dispersed in pollinia. The P/Os of 10 Asclepiadaceae range from 3.8:1–18.4:1 (Ali and Ali 1989, Cruden 1977, Wyatt and Broyles 1992). In most of these species each pollinium contained one to two pollen grains for each ovule in a carpel or ovary. The P/Os of the few orchids that have been determined were uniformly low ($n = 8$; range 3.9:1–24:1) (Mehrhoff 1983, Neiland and Wilcock 1995). The pollen of these orchids was dispersed in massulae, rather than pollinia.

Several factors may contribute to the lower P/Os of plants whose pollen is dispersed in tetrads or larger units. First, there is an immediate pollen population effect. Second, just a single tetrad or polyad may be sufficient to produce a fruit (e.g. Cruden 1977, Snow 1986), and, in some, sufficient pollen grains to account for the fertilization of all the eggs (e.g. Hernández 1989, Knox and Kenrick 1983, Koptur 1984). Third, the cost of pollen production may be less because the plants produce fewer pollen grains and those may be smaller than in related species that produce monads, e.g. in *Xyris* (Cruden pers. obser.).

Both local mate competition (Queller 1984) and the reduction of sibling rivalry (Kress 1981, Uma Shaanker et al. 1988) were proposed as explanations for the low P/Os of species whose pollen grains are dispersed as polyads or pollinia. Both hypotheses were based on the observation that the number of pollen grains per pollen unit usually equaled or exceeded the number of ovules in an ovary or carpel (e.g. Cruden 1977), thus seeds in a developing fruit had a single father. However, in various taxa the number of ovules in an ovary greatly exceeded the number of pollen grains per pollen unit (Cruden 1997, Knudsen and Olesen 1993, Lloyd and Wells 1992, Neiland and Wilcock 1995), and the developing fruits were certainly multipaternal. In some *Acacia* some stigmas received more than a single polyad (e.g. Knox and Kenrick 1983) and multipaternal fruits were identified by using genetic markers (Muona et al. 1991). In *Calliandra* stigmas frequently received more than a single polyad and the eight pollen grains

in a polyad were less than the number of ovules per ovary in most species (Cruden 1977). Complete seed set in such species (Cruden et al. 1976) was evidence of multipaternity. Finally, multipaternity is possible in those Asclepiadaceae in which pollen tubes from a single pollinium may end up in both carpels (see Kunze 1991, Kunze and Liede 1991) and those orchids whose stigmas regularly receive more than a single pollinium (e.g. Proctor and Harder 1994).

Secondary pollen presentation

In plants with primary pollen presentation pollen grains move directly from the anther to the pollen vector and in those with secondary pollen presentation the pollen grains are placed on another flower part and from there they move to the pollinator. Secondary pollen presentation occurs in ca. 25 families (Yeo 1993). It is a family trait in Asteraceae, Campanulaceae, Lobeliaceae, and Goodeniaceae, and is a common trait in other families, e.g. Fabaceae, Proteaceae, and Rubiaceae. Howell et al. (1993) suggested that plants with secondary pollen presentation enjoyed a selective advantage due to precise placement and receipt of pollen. Such was not the case in many bird-pollinated *Grevillea* (Proteaceae), which place the pollen widely over a bird's breast (Lyon and Cruden pers. obser.) and various Asteraceae, Goodeniaceae, Campanulaceae, and Lobeliaceae (Cruden pers. obser.), or in wind-pollinated *Hippophae rhamnoides* L. (Kerner 1897). Most of the species in these families with low P/Os also had large stigmas. Finally, taxa that are models for the precise placement of pollen do have low P/Os but they also have large stigmas and primary pollen presentation, e.g. *Stylidium* and orchids.

Based on a limited data set Yeo (1993, p. 220–221) suggested that the P/Os of species with secondary pollen presentation might be lower than those of species with primary pollen presentation, thus more efficiently pollinated than those with primary pollen presentation. The suggestion is not supported by the data

available today. There was little difference between the P/Os of homoeious species with primary (median = 3546:1, quartiles = 1173, 8130) and secondary pollen presentation (median = 3220, quartiles = 2037, 5062) ($t = 0.329$; $N = 239, 71$; $p > 0.5$). Yeo's hypothesis merits further testing because a majority of the species with secondary pollen presentation were in Asteraceae ($n = 28$) and Fabaceae ($n = 29$) (see Cruden 1977, Cruden and Miller-Ward 1981, Lawrence 1985, López et al. 1999, Mejias 1994, Short 1981) and four other families (see Cruden 1997, Dahl 1989, Devlin 1989, Inoue et al. 1996, Lamont and Barrett 1988, Ohara and Higashi 1994, Ramirez and Seres 1994, Ramsey and Vaughton 1991).

Nectarless flowers

Various workers suggested that flowers that provide only pollen as a reward to foraging bees will produce larger numbers of pollen grains than species that provide nectar as a reward (e.g. Pellmyr 1985, Vogel 1978, but see Mione and Anderson 1992). The available data support the hypothesis ($t = 2.250$, $n = 66,124$, $p < 0.05$). Over 70% of the nectarless taxa were in *Solanum* ($n = 18$; Mione and Anderson 1992) and Fabaceae ($n = 29$; Cruden 1977, López et al. 1999), and 26 of the nectarless taxa were buzz-pollinated. There was no difference in the P/Os of buzz- and non-buzz pollinated nectarless flowers ($t = 0.314$, $n = 26,40$, $p > 0.5$). With respect to the reward bees receive from nectarless flowers, it is surely the biomass of pollen not the number of pollen grains that is important. The hypothesis merits additional study because there is no data on the biomass of pollen produced by related nectarless and nectariferous flowers.

Pollen number and quality: environmental factors

Any factors that affect the nutritional status of a plant may affect the amount and quality of pollen produced. Both pollen production and

pollen size decreased in response to the loss of leaves to herbivores (Frazee and Marquis 1994, Lehtilä and Strauss 1999, Quesada et al. 1995). Pollen quality as measured by pollen tube growth rates and seeds sired was negatively affected by herbivory (Quesada et al. 1995, Mutikainen and Delph 1996, also see review by Delph et al. 1997). In *Alstroemeria* leaf removal resulted in smaller pollen grains whose pollen tubes grew at a slower rate, aborted more frequently and sired fewer seeds (Aizen and Raffaele 1998).

A number of experiments indicate that low nutrient levels may negatively affect pollen number and/or size as well as pollen tube performance. High nitrogen and phosphorus levels were positively correlated with pollen grain number and size in zucchini (Lau and Stephenson 1993, 1994). In addition, pollen from plants in the high nutrient treatments sired more seeds, which was a consequence of faster pollen tube growth. Likewise, in *Clarkia unguiculata*, both pollen and ovule numbers were positively related to nutrient levels (Vasek et al. 1987) but not the P/O (also see Travers 1999). In wild radish the competitive ability of pollen tubes from plants grown in low nutrient conditions was reduced but not pollen grain number or size (Young and Stanton 1990b).

Further, damage to flowers by herbivores may reduce pollen available to pollinators (Krupnick et al. 1999) and/or reduce pollinator visits to both damaged and undamaged flowers (Strauss 1997, Krupnick and Weis 1999, Krupnick et al. 1999).

Pollen protection

Pollen that is exposed to dew or rain may lose proteins and other compounds that render it inviable, thus adaptations that provide protection to such pollen would have a direct and positive impact on fitness. This aspect of pollen biology was discussed at length by Kerner (1897, Vol. 2: 104–129) but has attracted little attention by modern workers. Adaptations that protect pollen from exposure to dew or rain include anthers that close when exposed to dew or rain,

e.g. in *Mirabilis nyctaginea* (Michx.) MacM. (Cruden 1973), *Lilium philadelphicum* L. (Edwards and Jordan 1992), *Bulbocodium*, *Alchemilla*, and various Lauraceae (Kerner 1897), and flowers that close at night or in response to cloudy and rainy weather, e.g. *Nemophila menziesii* (Cruden 1972), *Sanguinaria canadensis* L. (Lyon 1992), *Hepatica*, *Crocus*, *Eranthus* (Kerner 1897), and *Tulipa* (Cruden pers. obser.). In *Hepatica* and *Eranthus* the sepals increase in length and provide continuing protection to the pollen in anthers whose stamens have elongated (Kerner 1897). Kerner also suggested that such traits as pendulous flowers, flowers that are sheltered beneath leaves or bracts, and anthers that are hidden by other floral structures protect both the anthers and the pollen (see Eisikowitch and Woodell 1974). In wind-pollinated *Hippophae rhamnoides* L. the pollen was deposited on the bottom of the flower from whence it was subsequently dispersed. Prior to dispersal the pollen was protected by the sepals (Kerner 1897). It is unclear whether some of the adaptations Kerner discusses are adaptations in response to rain or dew or whether protection of the pollen is a secondary benefit from an adaptation to some other selective pressure, e.g. pollinators. Kerner's observations provide numerous hypotheses that should be tested (also see Dafni 1996).

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Abiotic pollen and pollination: ecological, functional, and evolutionary perspectives

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Abstract. The transport and capture of pollen in ~20% of all angiosperm families occurs in air and water. In other words, pollination is abiotic and occurs via the fluid media, not an animal vector. Whereas some early concepts considered abiotic pollination to be largely a stochastic phenomenon, there is sufficient evidence to indicate that wind pollination (i.e. anemophily) and water pollination (i.e. hydrophily) have deterministic features and are sophisticated fluid dynamic solutions to the problem of pollen release, dispersal, and capture.

An abiotic pollination syndrome is defined in which there is spatial or temporal separation of carpellate and staminate flowers, which are drab, a reduction in perianth parts, stigmas and anthers are exposed to the fluid, and typically unclumped pollen may be produced in large amounts. Separate pollination syndromes are defined for anemophilous (i.e. wind-pollinated), ephydriphilous (i.e. surface-pollinated), and hydrophilous (i.e. submarine-pollinated) plants. Distinctions are based on habitat and physical conditions for pollination, pollen size, shape, and ultrastructure, morphology and ultrastructure of stigmas, and outcrossing rates. For example, anemophilous pollen are spherical and small, ephydriphilous pollen are spherical or reniform and large, while hydrophilous pollen are filiform (i.e. filamentous) or functionally filiform. The pollination mechanisms and mechanics associated with these syndromes reveals a strong evolutionary relationship between plant morphology and fluid dynamics.

Key words: Anemophily, hydrophily, wind pollination, water pollination, biomechanics, fluid dynamics, ephydriphilous, hyphydrophily, diincliny, dichogamy, autogamy.

Whereas pollination biology has focused on biological vectors of pollen transfer, there has been an increased interest in abiotic pollination systems, in which pollen transfer occurs via physical (i.e. wind and water) rather than biological agents. This is especially true for water pollination. Unfortunately, a recent review of abiotic pollination is lacking. The purpose of this chapter therefore, is to: (1) examine the systematic distribution of wind and water pollination; (2) examine the pollen of wind- and water-pollinated plants; (3) examine the mechanisms of wind and water pollination; and (4) describe pollination syndromes for wind and water pollination.

Terminology

The terminology of abiotic pollination depends largely on the location of pollen transfer (Table 1). Delpino (as presented in Delpino and Ascherson 1871) recognized that pollen transfer could take place without animals and divided abiotic pollination into wind (anemophily) and water pollination (hydrophily)

Table 1. Abiotic pollination mechanisms in plants may occur through aerial and aquatic vectors, but the latter is restricted to the angiosperms (adapted from Ackerman 1995)

Environment Fluid	Agent or Mechanism of Pollination
(A) Aerial	1) Animal Vector 2) Wind (Anemophily) 3) Apomixis
(B) Aquatic (freshwater and marine)	1) Surface (Ephydrophily) <ul style="list-style-type: none"> (i) Anther/Pollen rafting to exposed stigmas (ii) Anther/Pollen rafting into meniscus around flowers 2) Underwater (Hyphydrophily) <ul style="list-style-type: none"> (i) Anther-meniscus shower (ii) Pollen shower (iii) Hydroautogamy and Hydrogeitonogamy <ul style="list-style-type: none"> 1. Internal via pollen and pollen tubes 2. External via air bubbles (iv) Water currents (Hydrophily) 3) Apomixis

categories. Whereas anemophily occurs in the air, hydrophily is more complicated as it depends on whether pollen transfer occurs on the surface or below the water. Delpino made this distinction and Knuth (1906) later provided the basis for the terms ephydrophily for surface pollination, and hyphydrophily for underwater pollination. Additional distinctions can be made for both ephydrophily and hyphydrophily, especially with respect to whether pollen and stigmas are wet (Les et al. 1997).

Systematic considerations

The diversity in abiotic pollination reflects the fact that anemophily and hydrophily have evolved many times in terrestrial and aquatic plants (Arber 1920, Sculthorpe 1967, Les et al. 1997, Niklas 1997, Raven et al. 1999). Anemophily is much more common than hydrophily, given that ~98% of the plants that pollinate abiotically are pollinated by wind (Faegri and van der Pijl 1979), as is reflected in the systematic survey presented in Table 2. Some of the earliest seed plants were pollinated by wind (Niklas 1992, 1997), as are present day

gymnosperms (e.g. conifers), catkin-bearing trees (e.g. Betulaceae, Fagaceae), and herbaceous plants including many important cereals (e.g. Poaceae, Cyperaceae) (see reviews in Faegri and van der Pijl 1979, Regal 1982, Whitehead 1983, Niklas 1985, 1992, Proctor et al. 1996). Whereas these plants are primarily (or obligatory) anemophilous, wind pollination is also found in taxa that are primarily entomophilous (i.e. facultative anemophilous). Indeed this is reflected in the recent pollination-biology literature, which reveals an increasing number of reports of anemophily in primarily entomophilous families (e.g. Berry and Calvo 1989, Dafni and Dukas 1986, Vroege and Stelleman 1990, Bullock 1994, Gomez and Zamora 1996, Goodwillie 1999). These studies indicate that entomophily can occur simultaneously with anemophily (i.e. ambiphily), under certain circumstances (e.g. Stelleman 1984). It may also be possible for insect-induced anemophily to occur, where insect movements within flowers trigger pollen release (Listabarath 1992).

A review of the angiosperm families presented in Table 2 indicates that anemophily

Table 2. Systematic distribution of abiotic pollination based on classifications by Cronquist (1988) and Vidakovic (1991)

Division	Subdivision	Class	Subclass [# orders]	Order	Family	Pollination Mechanism (ref)*
Pinophyta	Coniferophytina (Pinicae)	Ginkgoatae	Ginkgoales	Ginkgoaceae		anemophilous (2)
	Pinatae	Cordaitidae Pinidae (Coniferae)		Pinaceae		anemophilous (2)
				Taxodiaceae		anemophilous (2)
				Cupressaceae		anemophilous (2)
				Podocarpaceae		anemophilous (2)
				Cephalotaxceae		anemophilous (2)
				Araucariaceae		anemophilous (2)
				Taxaceae		anemophilous (2)
				Cycadaceae		anemophilous (2)
	Cycadophytina (Cycadicae)	Cycadatae		Stangeriaceae		anemophilous (2)
				Zamiaceae		anemophilous (2), entomophilous? (8)
				Gnetatae		
	Magnoliophyta	Magnoliopsida	Magnoliidae [8]	Welwitschiacea		anemophilous (2)
				Ephedraceae		anemophilous (2)
				Gnetaceae		anemophilous (2)
				Piperaceae		anemophilous? (4)
				Cabombaceae		anemophilous (1)
				Ceratophyllaceae		hydrophilous (11)
				Ranunculales		entomophilous, anemophilous (11)
				Hamamelidales	Platanaceae	entomophilous (4)
					Hamamelidaceae	anemophilous, (13, 15)
				Urticales	Ulmaceae	anemophilous (4)
					Moraceae	entomophilous, anemophilous, (4, 15)
	Juglandales			Urticaceae		
	Myricales			Juglandaceae		
				Myricaceae		

Table 2 (continued)

Division	Subdivision	Class	Subclass [# orders]	Order	Family	Pollination Mechanism (ref)*
Caryophyllidae [3]	Carophyllales	Fagales		Fagaceae		anemophilous (4), entomophilous (4)
Dilleniidae [13]	Malvales			Betulaceae		anemophilous (4)
	Violales			Achatocarpaceae		entomophilous, anemophilous (15)
	Salicales			Nyctaginaceae		entomophilous, anemophilous (15)
	Capparidales			Chenopodiaceae		anemophilous (4)
	Ericales			Amaranthaceae		entomophilous, anemophilous (15)
Rosidae [18]	Rosales			Polygonaceae		anemophilous (4)
	Santalales			Tiliaceae		anemophilous (1, 4), entomophilous (4)
				Flacourtiaceae		entomophilous, anemophilous (11)
				Cistaceae		entomophilous, anemophilous (15)
				Salicaceae		entomophilous, anemophilous (11)
				Capparaceae		anemophilous (4)
				Brassicaceae		entomophilous, anemophilous (15)
				Ericaceae		entomophilous, anemophilous (14)
				Rosaceae		anemophilous (11)
				Podostemales		entomophilous, anemophilous (11)
				Haloragales		anemophilous? (5)
				Rhizophorales		anemophilous (1)
						entomophilous, anemophilous (4)
						entomophilous, anemophilous (15)

	Loranthaceae	entomophilous, anemophilous (11)
Euphorbiales	Euphorbiaceae	entomophilous, anemophilous (11)
Sapindales	Sapindaceae	anemophilous (11) entomophilous (4)
	Aceraceae	entomophilous, anemophilous (11)
	Anacardiaceae	entomophilous, anemophilous (15)
	Rutaceae	entomophilous, anemophilous (15)
	Julianiaceae	entomophilous, anemophilous (15)
Celastrales	Aquifoliaceae	entomophilous (4), anemophilous (4)
Callitrichales	Callitrichaceae	anemophilous (1), hydrophilous (?6, 11)
Asteridae [11]	Hippuridaceae	anemophilous (1)
	Hydrostachyaceae	anemophilous (1)
	Plantaginaceae	anemophilous (4)
Plantaginales	Oleaceae	anemophilous (4, 15)
Scrophulariales	Scrophulariaceae	entomophilous, anemophilous (12)
Solanales	Polemoniaceae	entomophilous, anemophilous (16)
Asterales	Asteraceae	entomophilous, anemophilous (11)
Hydrocharitaes	Hydrocharitaceae	anemophilous (1), anemophilous (1, 4), epi-/hyphydrophilous (1)
Liliopsida		
Alismatidae [4]		
Najadales	Scheuchzeriaceae	anemophilous (1)
	Juncaginaceae	anemophilous (1, 4)
	Potamogetonaceae	anemophilous, ephydrophilous, hyphydrophilous (1)

Table 2 (continued)

Division	Subdivision	Class	Subclass [# orders]	Order	Family	Pollination Mechanism (ref)*
	Ruppiaceae					ephydrophilous, hyphydrophilous (1, 11)
	Najadaceae					hyphydrophilous (1)
	Zannichelliaceae					ephydrophilous, hyphydrophilous (1)
	Posidoniaceae					hyphydrophilous (1)
	Cymodoceaceae					ephydrophilous, hyphydrophilous (1)
	Zosteraceae					ephydrophilous, hyphydrophilous (1)
Arecidae [4]	Arecales					hyphydrophilous (1)
Commelinidae [7]	Commelinales					entomophilous, anemophilous (4)
	Eriocaulales					anemophilous (4), entomophilous (1, 4)
	Juncales					anemophilous (1, 4), entomophilous (4)
	Cyperales					anemophilous (1) anemophilous (1, 4), entomophilous (4)
Liliidae [2]	Liliales					anemophilous, entomophilous (4)
	Hydatellales					anemophilous (1)
	Typhales					anemophilous (4)
						anemophilous (11)
						entomophilous, anemophilous (9)

(1) Cook (1996), (2) Raven et al. (1999), (3) Smith (1977), (4) Zomlefer (1994), (5) Philbrick and Retana (1998), (6) Osborne and Philbrick (1994), (7) Cronquist (1988), (8) Norstog et al. (1986), (9) Dafni and Dukas (1991), (10) Vidakovic (1991), (11) Proctor et al. (1995), (12) Cox (1991), (13) Whitehead (1983), (14) Gomez and Zamora (1996), (15) Bullock (1994), (16) Goodwillie (1999).

has been reported in 60 families, which represents ~16% of the total families richness within the angiosperms according to Cronquist (1988; recognizing that recent classifications differ; e.g. Thorne 1992, Les et al. 1997). This diversity includes all subclasses of dicotyledons and monocotyledons, with the exception of the Zingiberidae. Patterns in the ordinal distribution of anemophily are less obvious, but anemophily is present in $\leq 50\%$ of the orders within a given subclass. A notable exception is the Commelinidae, where anemophily has been reported in $> 85\%$ of the orders. The distribution of families within orders is log-normally distributed, with the majority of orders containing zero, one, or two anemophilous families (Fig. 1). This supports the concept that anemophily has arisen multiple times. Exceptional cases include (1) the Utricales, Callitrichales, and Najadales, (2) the Carophyllales, and (3) the Sapindales, with three, four, and five anemophilous families, respectively. Unfortunately, it is not possible to examine the species richness within anemophilous families at present, although simple contrasts can be made between families with high and low numbers of anemophilous species (e.g. Poaceae and Aceraceae).

Hydrophily is found primarily in monocotyledons, which contain 80% of the families in which hydrophilous species have been reported, or 2.7% of all angiosperm families

(Table 2). The two dicotyledon families, Ceratophyllaceae and Callitrichaceae, are found in the Magnolidae and the Asteridae subclasses, respectively. The remaining eight families are all found within the Alismatidae, which is a well-known aquatic group (Tomlinson 1982), with the Hydrocharitales represented by a single family, and the Najadales containing the remaining seven hydrophilous families. The distribution of species within families is not uniform, as the three seagrass families (Posidoniaceae, Cymodoceaceae, and Zosteraceae) are exclusively hydrophilous, but the same cannot be said of the remaining families. The seagrasses comprise ~50 of the ~130 reported hydrophilous species (Cook 1996).

At least three of the hydrophilous families also contain species that are anemophilous. The association between aquatic plants and anemophily has long been recognized and was believed to be an adaptive characteristic (e.g. Sculthorpe 1967). Cook (1988) examined this relationship and found that 119 of the 380 aquatic angiosperm genera contain anemophilous species, the majority of which have close relatives that are anemophilous (e.g. 100 of 119), or are in exclusively anemophilous families (e.g. 16 of 119 genera). Two of the remaining three anemophilous genera appear to have been derived from terrestrial entomophilous taxa. The association between anemophily and aquatic plants thus appears to be due to systematic affinity, rather than selective pressures of the aquatic environment (Cook 1988).

A systematic survey reveals abiotic pollination in 14 gymnosperm families and 67 angiosperm families, the latter representing 18.3% of the total angiosperm families. This total is reasonably close to the 84 families estimated by Renner and Ricklefs (1995) from pollination studies and extrapolation from floral patterns. It would not be unreasonable to conclude that abiotic pollination is important in the angiosperms, as ~20% of all angiosperm families contain abiotically-pollinated species (~18% are anemophilous, and ~3% are hydrophilous).

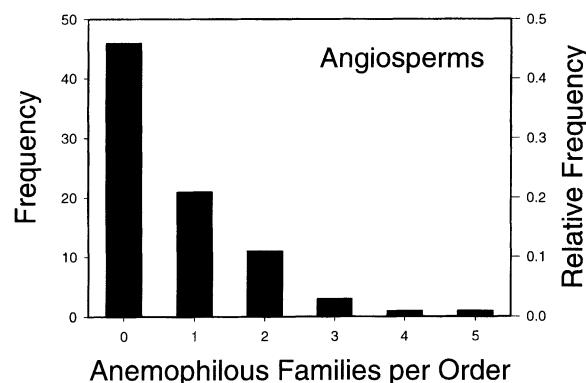


Fig. 1. Frequency distribution of the occurrence of anemophily in families within orders of angiosperms

Ecological and evolutionary considerations

Ecologically, anemophily is associated with either spatial or temporal separation of male and female reproductive structures (i.e. dioecy and dichogamy, respectively), which may, for example, encourage outcrossing (Charlesworth 1993, Renner and Ricklefs 1995). Results of genetic studies indicate that there is a dichotomy in the outcrossing rates of anemophilous plants, with either low rates (i.e. selfing) or high rates (i.e. cross pollination) (Schemske and Lande 1985, Barrett and Eckert 1990). This would indicate differences in the mating systems of different taxa. Among mating systems however, outcrossing-wind-pollinated species appear to have the highest rates of gene flow (Hamrick et al. 1995).

Atmospheric conditions influence pollen release, which usually occurs under dry conditions and before leafing, which are conditions that favour pollen dispersal (Whitehead 1983). Interestingly, researchers have examined the electrostatic attraction that can occur over short distances between positively charged pollen and negatively charged terminal ovulate and carpellate organs (Corbet et al. 1982, Gan-Mor et al. 1995, see Vaknin et al. 2000, this volume), although this has not been examined empirically in anemophily (e.g. Erickson and Buchmann 1983). There are other important geographic associations of anemophilous plants with certain environmental conditions including: low species diversity; a higher proportion of conspecifics; and dry conditions (e.g. high latitudes vs. tropical rainforests; Regal 1982, Whitehead 1983). However, the interpretation of the reasons for these associations has been called into question for ecological reasons (Midgley and Bond 1991a, b).

In hydrophily, as in the case of anemophily, there is a separation of carpellate and staminate reproductive structures. Hydrophilous flowers can be monoecious, dioecious, hermaphroditic, or dichogamous (phenological separation between pollen release and pollen reception) (Pettitt et al. 1981, Les et al.

1997). In the latter, protogyny appears to be more prevalent than protandry (Mahabale 1968). While these conditions have been interpreted to be due to selection for outcrossing in hydrophily, Les (1988) suggested that dicliny might be an ancestral condition. Nonetheless, these life history features would facilitate outcrossing by effectively separating the pollen and stigmas of the same flower or inflorescence. Importantly, Waycott and Sampson (1997) demonstrated that the outcrossing rates of the seagrass, *Posidonia australis*, are more similar to those observed in entomophilous plants (i.e. there was greater variability as compared to anemophily), and hydrophilous outcrossing rates depend on physical factors that influence pollen transfer (e.g. depth, currents). Pollen release must occur under conditions favorable for pollen transfer. In ephydrophilous plants, this corresponds to moderate flow conditions otherwise fertilization is reduced (Sullivan and Titus 1996). Conversely, in true hydrophilous plants, currents must be present for pollen to be released from anthers (Pettitt 1984, Verduin 1996, Ackerman 1997b).

There is a high degree of clonality associated with hydrophilous plants, but as in the case of several other generalizations, this appears to be due to the systematic affinity of the taxon (e.g. monocotyledons; Grace 1993). Specifically, clonal growth is quite common within the monocotyledons (Grace 1993). Lastly, the potential isolation within separate water bodies may have contributed to the multiple origin of hydrophily evident in this examination (Ackerman 1998).

Abiotic pollen

(1) Anemophilous plants

Traditionally, anemophily has been viewed as an inefficient process because pollen to ovule ratios in anemophilous plants are $>10^6:1$ (e.g. Faegri and van der Pijl 1979), e.g. $>10^{15}:1$ in *Ambrosia* (Payne 1981), and the aerodynamics

of pollination were not understood (e.g. Niklas 1985). Large pollen-ovule ratios are not unique nor are they representative of all anemophilous plants (see Cruden 2000, this volume). There can be little doubt, however, that relatively high pollen-ovule ratios would be advantageous in turbulent fluids where random or chaotic-like patterns are associated with particle motion within eddies. However, similarly high ratios may also be advantageous in biotic pollination given the vagaries associated with pollinators (Burd 1994). There may be other explanations for high pollen-ovule ratios in anemophilous plants. For example, Niklas (1992) interprets the large production of pollen in anemophilous plants to be related to the differences in metabolic costs relative to biotically-pollinated plants, which must invest energy to attract and usually reward pollinators. Moreover, Midgley and Bond (1991a) use ecological arguments that suggest that large pollen-ovule ratios are associated with intramale competition, which is consistent with field observations (e.g. Schoen and Stewart 1986, Honig et al. 1992).

Anemophilous pollen are small in diameter relative to biotically-pollinated plants (i.e. 20–60 µm vs. ≤200 µm; Harder 1998), and this is believed to reduce the settling velocity and, thus, increase the dispersal distance of the pollen (e.g. Niklas 1985, 1992). In some species of *Typha*, however, pollen released as tetrads germinate on stigmas once captured, and their pollen tubes may fertilize neighboring flowers (Nicholls and Cook 1986). Various mechanisms exist to increase dispersal distances by reducing pollen mass, and principal among them, are the air bladders (sacci) of many plants especially conifers. The traditionally held belief that saccate pollen of conifers function exclusively in this way (e.g. Crane 1986), has given way to the recognition that sacci are also important after airborne dispersal (e.g. Tomlinson 1994, Runions et al. 1999). The occurrence of saccate pollen is not uniform within conifers (i.e. many anemophilous conifers do not have saccate pollen), but it

does appear that sacci provide floatation on the pollination drop, which when absorbed draws the pollen to the micropyle (Tomlinson 1994). The sole exception appears to be the pollen of *Picea orientalis* that sink in the pollination droplet. This is unique in that the pollination droplet has an upright orientation in this species due to the pendant nature of the ovulate cones (Runions et al. 1999). Non-saccate conifer pollen are also wettable and sink and, either (1) burst within the pollination droplet of taxa with pollen droplets, or (2) do not burst and are drawn to the micropyle by extended siphonogamy or micropylar invaginations, depending on the taxon (Tomlinson 1994).

Anemophilous pollen are generally unornamented and do not contain pollenkitt, which eliminates the possibility of clumping (Crane 1986). Importantly, experiments have shown that clumped pollen have higher settling rates than unclumped pollen (Niklas and Buchmann 1988). There is a strong convergent tendency towards reduction in pollen apertures, which is thought to reduce water loss on exposure to air in both anemophilous (Crane 1986) and xerophytic plants (Thanikaimoni 1986). In the former, these are seen as shifts from tricolporate to triporate pollen and monosulcate to monoporate pollen, which function effectively to reduce the aperture to non-aperture ratios. However, dehydration does occur and results in some water loss, but not necessarily a major change in shape due to the smaller aperture sizes of anemophilous pollen (Crane 1986).

Lastly, Dafni and Firmage (2000, this volume) reviewed the subject of pollen longevity and demonstrated that anemophilous pollen had reduced longevity compared to entomophilous pollen. The pollen of entomophilous plants have pollen longevities that range from several days to almost several months. The reduction in pollen longevity of anemophilous plants is especially acute for species of Poaceae (e.g. <0.5 days), while reports in conifers are generally longer (e.g. several days).

(2) Hydrophilous plants

The pollen-ovule ratios of hydrophilous plants are not well characterized, but results indicate that the ratios are orders of magnitude less than anemophilous plants (Philbrick and Anderson 1987). While the sample sizes are small, freshwater plants have ratios between 10^2 and 10^3 (Philbrick and Anderson 1987), and the sole hydrophilous marine species that has been examined is on the order of 10^3 to 10^4 :1 (Ackerman 1993). The issue of pollen mass (i.e. size) is not important or relative to the issue of pollen dispersal, as settling in water is directly related to the density difference between the pollen and the water, not the mass (see Vogel 1994). Consequently, there is quite a range of pollen sizes and shapes in hydrophilous plants, with spherical, ellipsoidal, reniform (boomerang-shaped), and filiform (filamentous or conervoid) pollen present in various groups (e.g. Iwanami et al. 1988). In terms of size, the largest pollen is found in the seagrass *Amphibolis*, which has filamentous pollen 6 mm in length and ~ 20 μm in diameter (Ducker and Knox 1976). The functional aspects of filamentous pollen morphologies to pollination are discussed below.

The pollen of hydrophilous plants varies according to the type of pollination mechanism employed (see Table 1). In general, however, hydrophytic plants, including hydrophilous ones, have pollen with reduced exine, more apertures, and are consequently less rigid than the pollen of terrestrial plants (Pettitt and Jermy 1975, Pettitt 1984, Thanikaimoni 1986, Diez et al. 1988). Given that the polarity of these characters is opposite to what was observed in anemophilous plants, it is reasonable to suggest that dehydration is not a selective pressure acting on the pollen ultrastructure of hydrophilous plants. Rather, there are a number of potential waterproofing mechanisms in the pollen wall of hydrophilous plants to prevent them from bursting (Thanikaimoni 1986). Moreover, Thanikaimoni (1986) indicated that hydrophilous plants have omniaperturate pollen with thin elastic exine to

permit some increase in size. Nonetheless, hydrophilous pollen must adhere to stigmas, "hydrate", and undertake the appropriate biochemical recognition with stigmas, while submerged (Pettitt 1984). The difference between anemophilous and hydrophilous pollen can be seen between closely related families, where, for example, hydrophytic families have less ornamentation (e.g. Haloragaceae; Pvereen and Qaiser 1996). There are a number of similar contrasts that can be made within taxa that have terrestrial and aquatic species, such as the *Utricularia* and the *Callitriches*. In the latter, obligately submerged species are smooth and lack exine, while anemophilous species are ornamented with more well-developed exine (Martinsson 1993, Osborne and Philbrick 1994). Unfortunately, there are exceptions in some ephydrophilous taxa such as *Ruppia*, which has heavy reticulate exine that may help it float on the water surface (Lacroix and Kemp 1997). Floatation is also facilitated in a number of freshwater ephydrophilous taxa by starch grains in the pollen, which provide buoyancy for a given period of time (Mahabale 1968).

Ackerman (1995) demonstrated that true hydrophily, in which pollen is carried by water current in an analogous manner to anemophily (i.e. submarine pollination), is largely restricted to the marine monocotyledons or seagrasses. One of the most distinct and unusual features of seagrasses is that their pollen morphology is convergent and either filamentous (i.e. filiform) or functionally filamentous depending on the taxon (e.g. Najadales and Hydrocharitales, respectively; den Hartog 1970, Pettitt 1984, Tomlinson 1982, Ackerman 1995). In the latter, filamentous structures occur when: (1) the spherical pollen of *Thalassia* germinate precociously and/or travel in mucilaginous chains (Bowman 1922, Pascasio and Santos 1930); and (2) the linear tetrads of ellipsoidal pollen of *Halophila* travel within the same mucilaginous structure (Balfour 1879, Kausik and Rao 1942). *Halophila* is interesting in this case, as linear tetrad development, as opposed to a tetrahedral one (e.g. Pettitt 1984, Iwanami et al. 1988), can be considered a preadaptation to hydrophily.

Pollen morphology and development within the Najadales indicates that filiform pollen has evolved a number of times in different families (Ackerman 1995). Generally, the ultrastructure of the pollen reveals a reduction in the wall structure, which consists of two (or three) stratified microfibrillar layers in the Zosteraceae, slight stratification in the Cymodoceaceae, and no stratification in the Posidoniaceae (Pettitt 1984). There is also a reduction in exine ornamentation (Sculthorpe 1967, Pettitt and Jermy 1975), which is absent in the Cymodoceaceae (e.g. *Amphibolis*; Pettitt et al. 1978). The orientation of pollen within anthers is also dependent on systematic affinity. Pollen are oriented parallel to the long axis of the anther in the Zosteraceae (Dudley 1893, Rosenberg 1901, Pettitt and Jermy 1975, Ackerman 1993), and are either spirally or irregularly oriented within the Cymodoceaceae (Bornet 1864, Pettitt 1976, Yamashita 1976, Ducker et al. 1978). The strongest evidence for polyphyly of filamentous pollen stems from meiosis, which must ultimately lead to the elongation of microspores into filamentous pollen. This elongation of microspores occurs before reductive division in the Zosteraceae (Rosenberg 1901, Pettitt and Jermy 1975, Stewart and Rüdenberg 1980), but after reductive division in the Cymodoceaceae (Yamashita 1976, Pettitt 1981, 1984, Pettitt et al. 1981). As in anemophilous plants, pollen longevity in filamentous pollen, as indicated by cyclosis, is reduced compared to entomophilous plants. For example, cyclosis was only found in 10% of pollen after a day post anthesis, but cyclosis was still observed in a few pollen after two days (de Cock 1980). Filamentous pollen morphologies are convergent in the seagrasses and are an adaptation to submarine pollination (see below and Ackerman 1995, 1997a, b, 1998).

Pollination mechanisms

(1) Anemophilous plants

The majority of evidence on the mechanics of anemophily can be attributed to Niklas and

co-workers, who used helium bubbles and stroboscopic photography to visualize the flow and transport of pollen around ovulate and carpellate reproductive structures in wind tunnels (see reviews in Niklas 1985, 1992). The reproductive structures of wind-pollinated plants include strobuli (cones), catkins, and spikelets of grass flowers, which have brush or feather-like stigmas to aid in pollen capture (Niklas 1985, Crane 1986, Proctor et al. 1996). These reproductive structures are not showy (e.g. colorful perianths), nor do they contain the typical rewards (e.g. nectaries) found in biotically-pollinated plants. These generalizations do not, however, apply in all cases given the diversity and secondary derivation of abiotic pollination reported above. Niklas' efforts demonstrated that ovulate and carpellate structures, including in some cases, bracts and branches, modify airflow patterns in ways that favor pollination. Specifically, these include: (1) air flow that direct pollen to receptive structures; (2) downstream eddies that recirculate and redirect pollen to receptive structures; and (3) alternating air flow in downstream eddies that lead to the deposition of pollen on receptive structures (Niklas 1985). Consequently, pollen capture is favored under the fluid dynamic conditions found around ovulate and carpellate structures.

While the fluid dynamic evidence indicates that anemophily is a highly canalized process, empirical studies of the aerodynamic segregation of pollen among anemophilous species is even stronger evidence. For example, Niklas and Buchmann (1987) demonstrated striking pollen discrimination between pollen and ovules of two *Ephedra* species, which had very distinct pollen sizes. Their results and computer predictions were consistent with field observations, which included higher capture of conspecific pollen in pollination droplets (Buchmann et al. 1989). Recently, Linder and Midgley (1996) provided the strongest evidence for pollen discrimination in anemophilous plants, in their field study of four anemophilous species from four different families in South Africa. Their study revealed that

a minimum of 40% of conspecific pollen was found on stigmas (>80% in one case), even though stigmatic surfaces also contained alien pollen. This discrimination is remarkable in that it was disproportionate from the background pollen frequencies obtained on gel-coated slides. It is not clear whether similar discrimination would occur among more closely related species, say within a family. Niklas and Paw (1983) provide some information on this point, in that they found that pollination of pine species occurred at higher frequencies than pollination by congeneric pine species in reciprocal experiments conducted in the laboratory. In addition, there appears to be little clogging of foreign pollen on the stigmas of anemophilous plants (e.g. Honig et al. 1992). In summary, it appears that wind pollination is a highly canalized process involving the interaction of fluid dynamics and biological structures.

(2) Hydrophilous plants

Aquatic plants are taxonomically diverse and they are pollinated by a large number of aerial and aquatic mechanisms (Table 1; Arber 1920, Sculthorpe 1967, Faegri and van der Pijl 1979, Haynes 1988, Les 1988, Cook 1996, Proctor et al. 1996). Pollination in most aquatic plants, including submerged ones, occurs in the air either through biotic pollination or anemophily. As discussed above, the high proportion of anemophilous aquatic plants is due to systematic affinity rather than functional constraints (Cook 1988). Conversely, hydrophily is more limited (i.e. 18 or 19 genera; Cook 1988), and is categorized by the location of pollen transport. Ephydriophily is characterized by pollination on the water surface, in which surface and wind currents cause pollen and/or detached staminate flowers or anthers to "raft" to emergent or slightly submerged carpellate flowers and inflorescences. These flowers may be actinomorphic and white in color (e.g. Mahabale 1968). Note that an important distinction must be made as to whether the pollen is transferred wet or dry, as the latter

would necessitate considerable modification from an ancestral condition. Ephydriophily occurs when the pollen or stamens touch exposed stigmas, or when pollen or anthers dip into the meniscus around partly submerged carpellate flowers/inflorescences and are deposited on stigmas or shed pollen that drops onto stigmas (Arber 1920, Sculthorpe 1967, Cook 1982, 1988, Cox 1988). Cook (1982) provides an excellent review of these processes in freshwater plants. Svedelius (1932) proposed that wave action could lead to complete submergence of flowers, in which pollen contained in the meniscus would sink through the water towards stigmas. This "meniscus shower" may represent one step in the transition towards submersible pollen. Cox and co-workers (review in 1988) have expended considerable effort in applying Random Search Theory to ephydriophily. This mathematical model predicts: (1) Brownian recurrence in two dimensions; and (2) that the probability of a surface ship finding a submerged target is a function of the width of the ship's sonar beam (Koopman 1956). While the theory is valid, the application to surface pollination is not. The strongest argument against Random Search Theory is that, among other things, it assumes pollen transport to be random and, thus, recurrent. This is not the case, as wind generated and other surface movements are directional not random (also see Ackerman 1995, 1998b, Sullivan and Titus 1996, Verduin et al. 1996, Waycott and Sampson 1997).

Hypdrophily, or underwater pollination, is relatively uncommon in angiosperms, and largely restricted to the monocotyledons (Les et al. 1997). The most common mechanism in freshwater species is for pollen to "shower" down to enlarged stigmatic surfaces from elevated anthers (Sculthorpe 1967, Guo et al. 1990). Unfortunately the pollination mechanisms in most taxa remain to be described, although recent genetic data indicate that outcrossing does occur in submerged plants (see Osborne and Philbrick 1994). Hydroautogamy and hydrogeitonogamy occurs in both classes of angiosperms. In some cases, this is

an external phenomenon, in that pollen transfer takes place on the interface of air bubbles, which form around flowers or inflorescences (Philbrick and Anderson 1987). These bubbles likely originate from the lacunal gas system that are characteristic of aquatic plants (Sculthorpe 1967, Esau 1977). In other cases, autogamy and gietonogamy may be an internal event, where pollen may be released within floral buds, or more interestingly, when precocious pollen tubes penetrate ovules, sometimes after burrowing through vegetative tissues (Philbrick 1984). Internal geitonogamy through the latter mechanism is not an isolated phenomenon, as it has been recorded in seven species of *Callitricha* (Philbrick and Bernar-dello 1992).

True hydrophily, i.e. submarine pollination, in which pollination occurs through the action of water currents, occurs in the marine monocotyledons, or seagrasses (den Hartog 1970). It is not unusual, however, for intertidal seagrasses to pollinate via ephydrophily when they are exposed at low tides, but subtidal populations are common and exist at depths where only hydrophily is possible (see Ackerman 1998). Seagrasses are a functional group of ~50 species in twelve or thirteen genera and five families, which occur within three clades (Les et al. 1997). Regardless, they possess a number of morphological features that appear to be associated with hydrophily (Ackerman 1995). Specifically, their pollen is unique and has evolved convergently to filamentous shapes. As described above, filamentous pollen occur in the Najadales, and are created functionally in the Hydrocharitales (Pettitt 1984, Ackerman 1995). The female flowers of seagrasses are generally elongate stigmatic surfaces of highly reduced green-colored flowers, which, as in the case of anemophilous flowers, lack petals, nectaries or other features characteristic of biotically-pollinated plants (see drawings in den Hartog 1970). The stigmas are papillate and organized into ridges in the Hydrocharitales, and non-papillate and flat or bullate in the Najadales (Pettitt 1984). The seagrasses are found in marine coastal areas

throughout the world including Hudson's Bay (den Hartog 1970), where they are exposed to tidal currents and wind-generated waves (Nixon 1988).

A review of the pollination mechanisms of seagrasses is presented in Pettitt (1984). Unfortunately, these observations are limited taxonomically, and especially with respect to quantitative observation of the mechanics of submarine pollination. At present, the sole exception is the north temperate species, *Zoster* *marina*. The mechanics of pollination in this species was examined in the laboratory using stroboscopic photography and in the field using pollen models and gel-coated surfaces (Ackerman 1989, 1997a, b). Hydrophily appears to be governed by smooth and more viscous conditions (i.e. lower Reynolds number; see Niklas 1992, Vogel 1994), than the turbulent eddies described for anemophily. The emergence of female flowers from within the inflorescence, results in an increase in the shear stress (τ) in the local flow (Ackerman 1997a). When the filamentous pollen (2.7 mm × 7.5 μm) encounter this environment (i.e. higher τ), they rotate and cross streamlines towards female flowers, and thereby increase the opportunity for pollination (Ackerman 1997b). The axial force that causes these movements is directly related to the length and aspect ratio of the pollen (Forgacs and Mason 1958). Filamentous and functionally-filamentous pollen are unlike spherical pollen in that they need only be in vicinity of female flowers to pollinate. Pollination can occur through: (1) direct interception on stigmas; (2) rotation within $\frac{1}{2}$ a pollen length of stigmas; and (3) by being redirected through streamlines towards flowers (Ackerman 1997b). Field investigations confirmed the canopy flow conditions necessary for these observations (Ackerman and Okubo 1993). Moreover, the differential recovery of filamentous vs. spherical pollen models lend additional support to this biophysical phenomenon (Ackerman 1989). Observations of the submarine pollination of *Amphibolis* in the field are consistent with these predictions (Verduin

1996, Verduin et al. 1996). While the observations of the mechanics of hydrophily are limited taxonomically, the strong convergence of filiform pollen in seagrasses indicates that the mechanics of filiform pollen operate in similar ways in other taxa. Filiform pollen, therefore, represents a functional adaptation

for submarine pollination (i.e. true hydrophily).

Data on the fluid dynamic segregation of pollen by sympatric seagrasses species does not exist. However, genetic studies reveal that some seagrasses maintain relatively high out-crossing rates through hydrophily (Ruckles-

Table 3. Pollination syndrome for anemophilous, ephydriophylic, and hydrophilous (i. e. submarine-pollinated) plants. Characteristics followed by “?” are questionable

Pollination Mode	Pollen	Receptive Organs	Ecology
Anemophily (wind pollination)	<ul style="list-style-type: none"> – spherical and small – smooth surface – normal exine – reduction in apertures – may be saccate – non-clumping – may be produced in large amounts (?) – released from exposed anthers – short lived 	<ul style="list-style-type: none"> – cones, catkins, or spikelets – flowers imperfect – reduced perianths – drab, not-showy – stigmas exposed, long and feathery or brush-like – ovules reduced in number 	<ul style="list-style-type: none"> – diclinous, dioecious – mixed pollination – pollen release under dry conditions – associations with certain habitats (e.g. high altitudes and latitudes) (?) – high outcrossing rates (low in selfing species)
Ephydriophily (surface pollination)	<ul style="list-style-type: none"> – spherical or reniform – large size – exine present – may be ornamented – may contain starch – produced in smaller amounts (?) – released from detached and floating anthers 	<ul style="list-style-type: none"> – flowers small – may be white and actinomorphic – peduncle very long – may be imperfect and drab (?) 	<ul style="list-style-type: none"> – diclinous, dioecious – pollen should be released under moderate flows – associated with shallow freshwater habitats – clonal (?) – outcrossing rates (?)
Hydrophily (submarine pollination)	<ul style="list-style-type: none"> – varied in shape and size – mostly filamentous – smooth and unornamented – exine reduced or absent – omniaperturate – sticky surface – precocious germination – non-clumping – may be produced in large amounts (?) – released from exposed anthers 	<ul style="list-style-type: none"> – flowers imperfect – reduced perianths – drab, not-showy – stigmas exposed, long and slender, may be bifid – stigmas may be palpitate or non-palpitate – ovules reduced in number 	<ul style="list-style-type: none"> – diclinous, dioecious – hermaphroditism – protogyny – pollen only released under flowing conditions – associated with marine coastal habitats – may be clonal (?) – high outcrossing rates

haus 1995, Waycott and Sampson 1997). Importantly, high outcrossing rates are found under physical conditions where pollen dispersal is favored (e.g. exposed bays; Waycott and Sampson 1997). While there are a large number of similarities between wind and water pollination, observations and characterization of the mechanics are lacking for most taxa.

Pollination syndromes

Pollination syndrome for anemophilous, ephydrophilous, and hydrophilous (i.e. submarine-pollinated) plants are presented in Table 3. This represents an attempt to draw some generalizations from the data described above, and questionable ones are noted. There are some patterns that occur in all three categories, which include: (1) the production of pollen; (2) the exposure of anthers; (3) the exposure of stigmas; (4) the reduction in floral parts (with the exception of ephydrophilous); and (5) the dichrous nature of plants. Major differences among anemophilous, ephydrophilous, and hydrophilous plants pertain to: (1) pollen size; (2) pollen shape; (3) pollen ultrastructure; (4) stigma morphology; (5) stigma ultrastructure; (6) outcrossing rates; and (7) the particular habitats and conditions associated with these pollination modes. While the differentiating characteristics provide the basis for the delineation of three separate pollination syndromes, the common characteristics serve as the basis for a general definition for abiotic pollination. Regardless, it is evident from this review that abiotic pollination, namely anemophily and hydrophily, are more precise fluid dynamic processes, and not the wasteful and inefficient processes that they were once considered.

Ideas for future research

The strongest case for future research stems from realization that there is a lack of information on the mechanisms and mechanics of abiotic pollination. What is known, is

largely from a few well-defined model systems. So for example, we lack information on the mechanics of anemophily of catkin-bearing trees, and the hydrophily of the majority of aquatic plants that pollinate on the surface or underwater. Our scientific knowledge remains largely descriptive and anecdotal. While much of the future effort should be directed to careful fluid dynamic study, there is ample room for well-conceived laboratory and field study, as has been the case in recent studies. There can be little doubt that abiotic pollination remains a field that is rich in opportunity.

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Pollen nutritional content and digestibility for animals

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Abstract. This paper reviews the literature concerning digestion and nutrient content of pollen. Four topics are addressed in detail: 1) The mechanism of pollen digestion by animals; 2) The efficiency of mechanical and digestive removal of pollen content by various animals; 3) Range and taxonomic distribution of pollen nutrients, and 4) Adaptive hypotheses proposed to associate pollen chemistry with pollinator reward. Studies on the mechanism(s) of pollen digestion remain inconclusive, but suggest that differences in digestibility among pollen types may reflect differences in pollen wall porosity, thickness, and composition. Although hummingbirds reportedly digest pollen very poorly, most animals studied, including those that do not regularly consume pollen, can digest 50–100% of ingested grains. Overlooked and recent research of pollen protein content shows that pollen grains may contain over 60% protein, double the amount cited in some studies of pollen-feeding animals. Adaptive hypotheses that associate pollen starch and pollen caloric content with pollinator reward remain unsubstantiated when critically viewed through the lens of phylogeny.

Key words: Pollen chemistry, pollen digestion, pollination syndrome, palynology, bees, nutrition.

Pollen grain digestion

Pollen walls so resist decay and digestion that they often survive intact and recognizable for

millions of years in bog and sediment deposits. Their durability lends paleoecologists a valuable tool for reconstructing ancient floras and paleoclimates. Their durability also presents pollen-feeding animals with a substantial digestive challenge: How to access the nutrient-rich cytoplasm encased within the refractory pollen wall?

Generalized structure of pollen grains

The outermost layer of the pollen wall is the pollenkitt, a semi-solid coating comprised primarily of neutral lipids, hydrocarbons, terpenoids, and carotenoid pigments (Dobson 1988) (see also Nepi and Franchi, and Hesse, this issue). Inside the pollenkitt is the exine (thin or lacking in some monocots, Kress et al. 1978), an often intricately-ridged matrix of the complex carbohydrate sporopollenin. The exine greatly resists decay and digestion, but is commonly perforated by one-to-several pores or slits (germination pores) that lead to the inner wall layer, known as the intine. The intine, composed primarily of cellulose and pectin, also resists decay and digestion, and forms the final barrier to the nutrient-rich cytoplasm. Thus, any animal consuming pollen contacts pollenkitt nutrients through external probing of pollen grains or ingestion of pollen grains, but must penetrate or dismantle two resistant pollen wall layers in

order to access cytoplasmic nutrients. Six basic methods have been suggested for animals to extract pollen contents:

- I Crack open the pollen wall mechanically
- II Pierce the pollen wall with sharp mouthparts
- III Dissolve the pollen wall with enzymes
- IV Induce germination or pseudo-germination
- V Burst the pollen wall through osmotic shock
- VI Penetrate the pollen wall with digestive enzymes

The first three of these methods are rare, and the last three are difficult to distinguish. Some pollen-feeding beetles and micropterygid moths possess grinding mandibles said to function in grinding pollen (e.g. Meeuse 1961, Grinfel'd 1975, Crowson 1981, Barth 1985, Thien et al. 1985). We have been unable to find any study that demonstrates whole pollen entering and crushed pollen leaving the mandibles of any animal, however. Claims in the literature appear to originate from morphological analyses that correlate certain mandible structure with a pollen diet (e.g. Grinfel'd 1975, Crowson 1981, Mann and Crowson 1981, Thien et al. 1985). Mann and Crowson (1981) described the asperated or tuberculated mandibular mola of some primitive chrysomelid beetles as an adaptation to crushing pollen, a mechanism considered plausible, yet unsubstantiated, by Samuelson (1994). Studies of higher Chrysomelidae revealed unbroken pollen grains in the gut (Samuelson 1994). Mann and Crowson (1981) considered mandibular cracking of individual pollen grains to be common in beetles of the Nitidulidae, Cryptophagidae, Phalacridae, Anaspidae, Orsodacninae, Aulacoscelinae, Anthribidae, and Nemonychidae. Early claims that honey bees cracked pollen grains with their mandibles (e.g. Snodgrass 1925) were not borne out by subsequent analyses of pollen digestion (see below). Whitcomb and Wilson (1929) determined that honey bees could not generate sufficient crushing force with their mandibles to crack pollen grains.

Two studies of pollen-feeding beetles show that some beetles may crack pollen grains in

their digestive tract, rather than in their mouthparts. The beetle *Cyclocephala amazona* ingests both pollen and lignified trichomes from the inflorescence of the peach palm, *Bactris gasipaes* (Rickson et al. 1990). The trichomes, of no nutritive value, apparently act as gastroliths that crush pollen grains in the beetle's digestive tract. The boll weevil also breaks pollen grains, but by an undescribed method. Cate and Skinner (1978) found that pollen grains that passed through boll weevils in less than two hours were defecated whole, whereas pollen grains retained for longer periods of time were more likely to be fragmented.

Numerous species of thrips (Thysanoptera) feed on pollen (reviewed in Kirk 1984). The diameter of the pollen grains of most species (15–60 µm, Simpson and Neff 1983) exceeds the breadth of the maxillary feeding channel of most thrips. Rather than ingesting whole pollen grains, thrips position themselves in front of individual grains, pierce the exine with their mouthparts, and suck out the contents, leaving behind collapsed or air-filled grains (Kirk 1984, Grinfel'd 1959, cited in Kirk 1984). This process takes from 2–20 seconds per grain, depending on size of grain, temperature, and thrips species. Thrips apparently do not need to pierce grains at particular positions, such as a pore, but may have to pierce large grains at multiple positions in order to extract all of the contents (Kirk 1984).

The only other organisms reported to pierce pollen grains and extract their contents without ingesting the grains are some species of ceratopogonid flies (Downes 1955, De Meillon and Wirth 1989). Female Ceratopogonidae ("biting midges," "no-see-ums") typically feed on the blood or hemolymph of living mammals, birds, or insects prior to laying eggs. Females of *Atrichopogon pollinovorus*, in contrast, perch on an anther or stigma, press the tip of their rostrum against a pollen grain, and extract the pollen contents in ca. 6 seconds (Downes 1955). Downes reported *Atrichopogon* spp. consuming the pollen of *Lonicera* sp., *Crataegus* sp., and *Prunus lusitanica*. He fur-

ther observed that the ovaries of pollen-foraging *Atrichopogon* spp. were small and undeveloped, similar in appearance to blood and hemolymph-consuming species of ceratopogonids prior to a blood meal, leading him to conclude that pollen consumption served to mature ceratopogonid ovaries and oocytes. Although male ceratopogonids were often observed sipping nectar on the same flowers as females, they were never observed consuming pollen.

Some Collembola ("springtails") are the only animals known to fully digest the pollen wall. Scott and Stojanovich (1963) fed *Juniperus* pollen to the semi-transparent collembolan *Onychiurus pseudofimetarius* and observed that the pollen wall ruptured then completely disappeared during its passage through the digestive tract. Not all collembolans, however, possess this digestive ability. Pollen walls remained intact while passing through the gut of *Entomobrya socia* (Waldorf 1981).

The discrete methods of pollen nutrient extraction described above account for half of the methods hypothesized, but they apply to very few of the organisms known to digest pollen. Most animals are thought to extract pollen nutrients through pollen germination (or pseudo-germination), osmotic shock, and/or penetration of the pollen wall by digestive enzymes. Pollen grains of many species will germinate in a 10% sucrose medium in the presence of micronutrients such as boric acid. As pollen germinates, it releases proteins (Stanley and Linskens 1965) and free amino acids (Linskens and Schrauwen 1969). Similarly, pollen pre-soaked in a 10% sucrose medium then placed in acid may form instant pollen tubes (IPoTs, a process hereafter referred to as pseudo-germination), similar in appearance to naturally germinating pollen but arising more suddenly (Linskens and Mulleneers 1967). Most pollen-feeding animals sip nectar while ingesting pollen, and could retain pollen in a nectar-filled, weakly acidic portion of the digestive tract (Turner 1984). Thus, they may subject pollen to conditions in which it either germinates or pseudo-germi-

nates, thereby extracting both free amino acids and proteins without breaking or penetrating the pollen grain.

Pollen-feeding *Heliconius* butterflies add nectar to a collected pollen mass, churn the mass externally for several hours with their proboscis, and ingest pollen-derived nutrients without ingesting whole pollen grains (Gilbert 1972). These nutrients greatly increase egg production and longevity in *Heliconius*. The eucalypt nectar fly, *Drosophila flavohirta*, also gathers a large pollen load on the tip of its proboscis and agitates it, without ingesting the pollen grains (Nicolson 1994). Nicolson considered this to be a nutrient gathering strategy similar to *Heliconius*. In both cases, the nutrients reportedly become available through nutrient release of pollen grains undergoing germination, rather than through pollen grain degradation.

The syrphid fly *Cheilosia albiparsis* ingests both the pollen and nectar of its host plant, *Ranunculus repens* (Haslett 1983). The pollen remains intact as it passes through the oesophagus and crop. As pollen reaches the midgut, however, most grains extrude their cytoplasm through a germination pore in a manner reminiscent of germination or pseudo-germination (Haslett 1983). Most pollen grains are empty by the time they reach the hindgut. Pollen grains in the crop of the oedemerid beetle *Stenostoma coerulea* show similar cytoplasmic protrusions and are empty when they reach the hindgut (Crowson 1981). The honey possum, *Tarsipes rostratus*, digests 90–100% of the *Banksia* pollen it ingests (Turner 1984). Turner found that some pollen in the lower stomach showed cytoplasmic protrusions similar to germinating pollen tubes, but Richardson et al. (1986) did not observe such protrusions.

Various flower-visiting bird species ingest pollen incidentally or intentionally while imbibing nectar at flowers. A species of Darwin's finch, *Geospiza scandens*, feeds cactus (*Opuntia echios*) pollen to its offspring prior to the onset of seasonal rains, a time of good pollen availability but poor alternative forage (Grant

1996). Grant found that *G. scandens* (and the related *G. fortis*) digested approximately 90% of pollen grains consumed. She demonstrated that most *O. echios* pollen will germinate in a solution of *O. echios* nectar and water, and hypothesized that the simultaneous ingestion of *O. echios* pollen and nectar led to pollen germination and subsequent digestion. Grant (1996) found that 11.5% of pollen in the crop of nestlings showed signs of germination. In contrast, Paton (1981) found that *Banksia* pollen grains germinated in a sugar solution *in vitro*, but not when consumed with sugar solution by the New Holland Honeyeater, *Phylidonyris novaehollandiae*.

Pollen digestion has been studied most extensively in bees, but a complex and somewhat confusing set of interpretations has emerged. Adult bees possess a crop, in which nectar and pollen may mix, thus providing a pre-treatment that could lead to germination or pseudo-germination. The crop leads through the proventricular valve to the gut, a region that differs substantially in osmotic pressure from the crop. Thus, pollen consumed by adult bees is subjected to immersion in a sugar solution followed by an abrupt osmotic gradient. In contrast, larval bees have no crop. Pollen enters the gut without internal exposure to nectar sugars or sudden changes in osmotic pressure. The pollen consumed by larval bees, however, is part of a food provision including a large quantity of nectar. Thus, larval pollen has been exposed to a liquid sugar environment prior to ingestion. For most bee species, larvae consume the pollen within a few days of pollen collection. For honey bees, however, pollen may be stored in the hive for an extended period of time. Adult honey bees add secretions that inhibit pollen germination (Klungness and Peng 1983). Additionally, the stored pollen undergoes considerable biochemical change due to the activities of microbial organisms. Thus, there may be differences in pollen digestion between larvae and adult bees, and among bees that differ substantially in sociality.

Kroon et al. (1974) noted that pollen grains will burst when transferred suddenly

between chambers that differed in osmotic pressure. These researchers determined that the osmotic differential between the honey bee crop and ventriculus was sufficient to burst pollen grains, and suggested that the burst grains then traveled through the gut where their contents were accessible to digestive enzymes. They hypothesized that this "osmotic shock" was a necessary prerequisite to digestion. Peng et al., however, (1985, 1986) did not find burst pollen grains in the anterior midgut of the honey bee. Instead, these researchers found that *Medicago sativa* (alfalfa) and *Taraxacum officinale* (dandelion) pollen slowly degraded and lost their contents during their passage through the midgut. *Taraxacum* pollen swelled at the germination pores in the bee's anterior midgut, but showed no further signs of germination (Peng et al. 1985). The pollen walls of *Taraxacum* gradually became misshapen as the cytoplasm drained through the germ pores. *Medicago* pollen showed germination-like cytoplasmic protrusions, still contained by the bulging intine, in the middle midgut (Peng et al. 1986). As the *Medicago* pollen reached the posterior midgut, the pollen wall partially broke down and the protruding intine ruptured, releasing the cytoplasm. Peng et al. (1986) hypothesized that the digestion process comprised enzymatic degradation of the pollen wall, followed by extrusion of the cytoplasm due to osmotic pressure, weakening of the intine by proteases, and eventual rupture of the intine by continued osmotic pressure. In this interpretation, cytoplasmic protrusions resembling germination are caused by cell wall breakdown and osmotic pressure rather than induction of germination. Although pollen wall components such as sporopollenin and cellulose are unlikely to be digested by honey bee digestive enzymes, Klungness and Peng (1984) found that honey bees could partially digest the hemicellulose and pectic acid components.

Cruz-Landim and Serrao (1994) and Serrao and Cruz-Landim (1996) studied pollen digestion in adult and larval stingless bees of the genera *Trigona* and *Scaptotrigona*. With

both larvae and adults, they found that pollen contents were digested without bursting of the exine. They inferred pollen digestion to occur as the result of bee digestive enzymes altering the pollen wall, followed by penetration into the pollen grain by digestive enzymes, then the bursting of the intine and release of pollen contents through osmotic pressure.

Suárez-Cervera et al. (1994) studied pollen digestion by larvae of solitary bees in the genus *Osmia*. They compared pollen from unconsumed portions of the provision mass with pollen consumed and defecated by mature larvae. The pollens with thin intines showed cytoplasmic protrusions at the germination pores in the provision mass (*Cistus* sp. and *Sonchus* sp.); these were better digested than pollens with relatively thick intines that showed no modification while in the provision (*Prunus* sp. and *Quercus* sp.).

Dobson and Peng (1997) studied pollen digestion in 2–3 week old larvae of the solitary, pollen-specialist bee, *Chelostoma florisomne*. By staining serial gut sections for protein, carbohydrates and lipids, Dobson and Peng determined the extent and location of digestion, as well as the condition of the pollen wall as it passed through the digestive tract. They found no evidence of pollen germination. They found that pollen grains gradually emptied their contents through the germination pore, without breaking their walls before losing most of their contents.

It is difficult to assemble all of these observations into a general mechanism of pollen digestion because different observations have been made on different organisms and different life stages with different pollens. No studies have been carried out that intentionally varied pollen characteristics in order to assess their effect on digestion. Dobson and Peng (1997) provide a lucid synthesis of pollen digestion studies on bees, which we will briefly summarize here. The intine that covers germination pores provides the greatest barrier to penetration of the pollen grains by insect digestive enzymes. For larvae of bee species other than honey bees, this layer may be

disrupted by germination-inducing conditions in the sugary pollen provisions. For bee adults, this layer may be disrupted at the germination pores by osmotic shock as the pollen passes from the crop through the proventricular valve. As the pollen passes through the gut, digestive enzymes degrade the hemicellulose and pectic acid components of the intine, further allowing pollen grain contents to flow out as insect digestive enzymes flow in. The digestion of particular compounds, such as starch, appears to depend on their removal from the pollen grain. Reported discrepancies regarding the extent to which pollen walls are broken probably arise from different chemical characteristics of the walls of different pollens.

Researchers studying pollen digestion by animals generally attribute changes in the pollen wall, aside from a slight swelling at the germination pores, to the action of animal-derived digestive enzymes. Pollen cell walls may be altered by pollen enzymes already present in the cell wall, however. During the initial stages of germination, the sporopollenin of the spines of *Pharbitis nil* pollen degrades into an amorphous layer (Gherardini and Healey 1969). Gherardini and Healey (1969) report on studies that show that hydrolytic enzymes are present in the intine and are particularly active near the germination pores. When pollen is placed on a stigma, even a non-congeneric stigma, cell wall degradation may begin without the appearance of a germination tube or other signs of germination. Thus, an absence of overt signs of pollen germination in the crop or gut of a pollen-feeding animal does not necessarily mean that important endogenous changes in pollen wall chemistry associated with germination-induction conditions have not already occurred.

Grain emptying efficiency of various animals

Digestive efficiency has been calculated as percent empty pollen grains in the feces of numerous animals (Table 1). Some researchers adjusted the efficiency to accommodate the percentage of empty grains pre-ingestion,

Table 1. Comparison of percent of pollen grains emptied by passing through the digestive tract of various animals

Animal group	Animal species	%empty	Plant species	Reference
Vertebrates				
Mammals				
Rodents				
	<i>Aethomys namaquensis</i>	60.4		Vantets 1997
	<i>Elephantulus edwardsii</i>	50.3		Vantets 1997
	<i>Mus minitoides</i>	83.0		Vantets 1997
	<i>Rhabdomys pumilo</i>	60.4		Vantets 1997
Bats				
	<i>Syconycteris australis</i>	53–57	<i>Banksia</i>	Law 1992b
	<i>Sturnira lilium</i>	47	columnar cactus	Herrera and Martínez del Rio 1998
		27	<i>Pseudobombax ellipticum</i>	
	<i>Artibeus jamaicensis</i>	71	columnar cactus	Herrera and Martínez del Rio 1998
		53	<i>Pseudobombax ellipticum</i>	
		32	<i>Hylocereus undatus</i>	
	<i>Anoura geoffroyi</i>	75	columnar cactus	Herrera and Martínez del Rio 1998
		82	<i>Pseudobombax ellipticum</i>	
		50	<i>Hylocereus undatus</i>	
	<i>Leptonycteris curasoae</i>	86	columnar cactus	Herrera and Martínez del Rio 1998
Marsupials				
	<i>Acrobates pygmaeus</i>	78	<i>Eucalyptus</i> sp.	Turner 1984
	<i>Petaurus australis</i>	60–70	<i>Banksia/Eucalyptus</i>	Quin et al. 1996
	<i>Tarsipes rostratus</i>	78.6	<i>Banksia integrifolia</i>	Turner 1984
		65.0	<i>Banksia serrata</i>	Turner 1984
		63.7	<i>Banksia spinulosa</i>	Turner 1984
Birds				
	<i>Pholidonyris novaehollandiae</i>	0		Paton 1981
		41 ^a	<i>Banksia/Dryandra</i>	Wooller et al. 1988
	<i>Melopsittacus undulatus</i>	42 ^a	<i>Banksia/Dryandra</i>	Wooller et al. 1988
	<i>Poephila guttata</i>	41 ^a	<i>Banksia/Dryandra</i>	Wooller et al. 1988
	<i>Geospiza scandens</i>	>90 ^a	<i>Opuntia echios</i>	Grant 1996
	<i>Geospiza fortis</i>	>90 ^a	<i>Opuntia echios</i>	Grant 1996
	<i>Calypte anna</i>	3.8	<i>Zauschneria</i> sp.	Brice et al. 1989
		4.7	<i>Callistemon</i> sp.	Brice et al. 1989
		6.9	<i>Eucalyptus</i> sp.	Brice et al. 1989
	<i>Calypte anna</i> (nestling)	5.4	<i>Eucalyptus</i> sp.	Brice et al. 1989
	<i>Calypte costae</i>	0	<i>Zauschneria</i> sp.	Brice et al. 1989
		0	<i>Eucalyptus</i> sp.	Brice et al. 1989
	<i>Trichoglossus haematodus</i>	4.5	<i>Eucalyptus</i> sp.	Brice et al. 1989
	<i>haematodus</i>			

Table 1 (continued)

Animal group	Animal species	%empty	Plant species	Reference
	<i>Trichoglossus h. moluccanus</i>	6.6	<i>Eucalyptus</i> sp.	Brice et al. 1989
		12.9	<i>Prunus</i> sp.	Brice et al. 1989
	<i>Trichoglossus h. m.</i> (nestling)	26	<i>Eucalyptus</i> sp.	Brice et al. 1989
		12.3	<i>Prunus</i> sp.	Brice et al. 1989
	<i>Nymphicus hollandicus</i>	18.1	<i>Eucalyptus</i> sp.	Brice et al. 1989
	<i>Nymphicus hollandicus</i> (nestling)	38.0	<i>Eucalyptus</i> sp.	Brice et al. 1989
Invertebrates				
Bees				
	<i>Apis mellifera</i> (adults)	50		Cruz-Landim 1985
	<i>A. mellifera</i> (1 day old adults)	98.2 ^a	<i>Castanea</i>	Crailsheim et al. 1992
	<i>A. mellifera</i> (1 day old adults)	72.6	<i>Trifolium</i>	Crailsheim et al. 1992
	<i>A. mellifera</i> (9 day old adults)	96.0 ^a	<i>Castanea</i>	Crailsheim et al. 1992
	<i>A. mellifera</i> (9 day old adults)	93.1	<i>Trifolium</i>	Crailsheim et al. 1992
	<i>A. mellifera</i> (23 day old adults)	49.5 ^a	<i>Castanea</i>	Crailsheim et al. 1992
	<i>A. mellifera</i> (23 day old adults)	62.0	<i>Trifolium</i>	Crailsheim et al. 1992

^a (%empty grains in feces – %empty grains in pollen prior to consumption)/%full grains prior to consumption

which can be substantial (e.g. Paton 1981), while other researchers did not account for this factor. In Table 1 we report digestive efficiency adjusted for pre-ingested grains whenever sufficient data were available. Efficiency ranged from nearly zero for some bird species (hummingbirds, *Calypte* spp., and the honey-eater *Phylidonyris novaehollandiae*) to 80–100% for some bat species, a pollen-feeding Darwin's finch, and the mouse *Mus minitoides* fed a laboratory diet of pollen. Honey bees extracted pollen contents from 50–98% of ingested pollen, depending on pollen type and age of the bee. Table 1 shows that various facultative pollen-feeders can extract the cytoplasm of more than half of the pollen grains they consume. Digestive efficiencies among pollen-feeders consuming different pollens cannot readily be compared because of differences in the pollen consumed. The bat *Artibeus jamaicensis* extracted the cytoplasm of 72% of the pollen grains of an unknown columnar cactus, but only 32% of the cytoplasm of the pollen grains from the cactus *Hylocereus undatus* (Herrera and Martínez del Rio 1998). It is interesting to note that some animals for

which pollen might not be a regular part of the diet, such as mice and some bats, can efficiently extract pollen cytoplasm. Herrera and Martínez del Rio (1998) found, however, that bat species that regularly consume pollen can digest it more efficiently than bat species that do not regularly consume pollen.

Nutritional content of pollen

Various authors reviewing pollen chemistry have reported the known range or average concentration of nutrients in pollen grains (e.g. Lundén 1956, Barbier 1970, Stanley and Linskens 1974, Solberg and Remedios 1980, Nepi and Franchi, this volume). Nutrients reported include protein, nitrogen, amino acids, starch, sterols, and lipids. Most analyses and summaries are based on few taxa [the most extensive survey cited usually being that of Todd and Bretherick's (1942) analysis of 31 plant species]. Chemical analyses of pollen are difficult because most analytical techniques require more pollen that can be easily collected by hand. Thus, two categories of plants have generally been targeted for analysis: 1) an-

mophilous (wind-pollinated) trees, such as *Pinus* and *Quercus*, that produce abundant pollen, and 2) entomophilous (insect-pollinated) or anemophilous species whose pollen is readily collected by honey bees and subsequently removed from honey bees by researchers (e.g. Todd and Bretherick 1942, Vivino and Palmer 1944, Standifer 1966, Ibrahim 1974, Standifer et al. 1980, Rabie et al. 1983, Schmidt and Johnson 1984). This has led to a good understanding of the pollen chemistry of a small number of anemophilous trees and a very imperfect understanding of the composition of honey bee-collected pollens. Honey bees mix pollen with regurgitated nectar or honey for transport on their legs. Thus, the "pollen pellets" often subjected to chemical analyses contain both pollen and nectar or honey. The fraction of pollen pellets attributable to added nectar or honey has never been estimated directly. The data set of Todd and Bretherick (1942) comprised both hand and honey bee-collected samples, including one species, *Pinus contorta*, that was collected by both techniques. Their work indicates that most reducing sugars present with pollen transported by honey bees are derived from added nectar or honey; these reducing sugars may account for up to 40% of the dry weight of pollen pellets ($\bar{x} = 25.7 \pm 5.19\%$, $n = 26$, honey bee-collected, $\bar{x} = 2.59 \pm 3.59\%$, $n = 6$, hand-collected). Because the chemical constituents of pollen grains are generally reported on a per weight basis, any analysis of pollen pellets that does not account for the added weight of nectar or honey sugars will greatly underestimate the concentration of the chemical constituents in the pollen itself. Comparisons of pollen protein estimates from nine species analyzed from both hand and honey bee-collected pollens led Roulston et al. (in Press) to conclude that the bias created by added sugar was extremely variable and could not be removed by a standardized multiplier. At present, we know nothing about the factors governing the amount of sugar honey bees add to transported pollen (i.e. if it depends on the properties of the pollen or merely depends on

the sugar concentration of the nectar or honey in the honey bee's crop). Most chemical analyses of pollen are based on honey bee-collected pollen. Thus, there is great uncertainty for the majority of reported values. While we may know the concentration of pollen constituents to the correct order of magnitude (assuming that none of the nutrients originate from nectar or the honey bee itself, as in reducing sugars), we often do not have sufficient data to compare chemical constituents across taxa or pollination modes for pollen that is taken from foraging honey bees.

Pollen nitrogen/protein content

Numerous studies have estimated the nitrogen/protein content of pollen (e.g. Lidforss 1899, Todd and Bretherick 1942, Nielsen et al. 1955, Levin and Haydak 1957, Knight et al. 1972, Howell 1974, Loper and Berdel 1980, McCaughey et al. 1980, Solberg and Remedios 1980, Standifer et al. 1980, Rabie et al. 1983, Buchmann 1986, Schmidt et al. 1987, Roulston et al., in Press). Data from hand-collected samples indicate that pollen ranges from 0.36% N for *Cupressus arizonica* to 9.7% N for *Dodecatheon clevelandii* (Buchmann 1986). The two most commonly-used techniques for estimating nitrogen, micro-Kjeldahl acid digestion and combustion, generally produce similar results (Buchmann 1986, Roulston et al., in Press).

Most estimates of crude protein concentration in pollen are derived from estimates of nitrogen concentration multiplied by 6.25. This conversion factor is well established for estimating animal protein mass from tissue such as muscle, but may not be fully accurate for plant tissues. Milton and Ditzis (1981) calculated a nitrogen-protein conversion factor of 4.4 for tropical leaves eaten by howler monkeys. Solberg and Remedios (1980) calculated conversion factors of 4.0 and 3.8 respectively for hand-collected and bee-collected pollens, but Rabie et al. (1983) suggested that those conversion factors failed to adequately

account for ammonia nitrogen. Rabie et al. (1983) analyzed honey bee-collected pollen from 21 plant species and suggested using a standard conversion factor of 5.6. Preliminary work with hand-collected pollens indicated that the conversion factor may be as low as 4.5–5.25 (Buchmann 1986). Thus, the common use of a 6.25 conversion factor may overestimate actual crude pollen protein concentration, but should provide accurate relative estimates when comparing plant species that have been analyzed by comparable techniques. Roulston et al. (in Press) showed that a modified Bradford Assay using *Typha latifolia* pollen as a protein standard produced similar pollen protein concentration estimates to those from micro-Kjeldahl and combustion.

Using the 6.25 conversion factor for the subset of raw N values, Roulston et al. (in Press) compiled a data base of pollen protein concentrations from 377 plant species in 93 plant families. They found that pollen protein concentration ranged from 2.5–61% protein and was highly conserved within plant genera and families. The plants poorest in pollen protein were anemophilous gymnosperms, such as Cupressaceae and Pinaceae, while those richest in protein were vibratile-pollinated herbs such as *Dodecatheon* (Primulaceae), *Rhexia* (Melastomataceae), and *Solanum* (Solanaceae), as well as bat-pollinated Bombacaceae and bird-pollinated Campanulaceae. Although many anemophilous pollens were protein poor, evolutionary shifts to anemophily were not statistically associated with decreased protein concentration (Roulston et al., in Press). Roulston et al. found no evidence that bee species preferentially collected pollen that was particularly rich in protein.

Animals collect pollens that may differ widely in protein content. Protein concentration may influence the frequency or absolute number of foraging trips an animal must make in order to provide offspring with sufficient pollen to meet their dietary protein requirements (Danforth 1990, Neff and Simpson 1997, Roulston and Cane submitted). Although some authors have stated that crude

protein estimates do not measure pollen quality (e.g. Todd and Bretherick 1942), no other chemical constituent of pollen grains has been shown to influence as many aspects of a pollen consumer's performance as well as protein. Levin and Haydak (1957) transplanted eggs of the solitary bee *Osmia lignaria* onto honey bee-collected pollens held in separate vials. Of the ten pollens tested, only the five richest in protein supported development to the adult stage. Diet supplementation with vitamin-free casein, yeast, and egg yolk indicated that protein, B-vitamins, and sterol content all influenced bee development. Schmidt et al. (1987) fed 25 pollens to adult honey bees and found that longevity increased with protein concentration of the pollen and total protein consumed. As noted by Schmidt et al. (1987) and Roulston and Cane (submitted), bees do not collect/consume equal amounts of all pollens. Thus, total protein consumed reflects both the protein concentration of the pollen and the amount of pollen consumed. Bees apparently do not prefer to collect protein-rich pollens (Van Der Moezel et al. 1987, but see Schmidt and Johnson 1984).

Bee body size varies with protein consumed. Regali and Rasmont (1995) found that larval nestmates fed one pollen diet by worker bumble bees grew larger than those fed a second pollen diet that was poorer in protein. Greenberg (1982) and Roulston and Cane (submitted) increased the size of *Lasioglossum zephyrum* offspring by supplementing an adequate but protein-poor diet of *Typha latifolium* pollen with soy protein powder. Levin and Haydak (1957, data plotted from Levin and Haydak's Table 4) and Roulston and Cane (submitted) showed that adult bee body size increased linearly with total protein consumed.

Atypical of bee species, honey bee workers monitor the growth of developing larvae and feed them periodically with a diet rich in glandular secretions that originate in the hypopharyngeal glands. The hypopharyngeal glands develop to a great extent after adult emergence, reaching their maximum size after five or more days (Winston 1987). Standifer

et al. (1967) found that the development rate of worker hypopharyngeal glands increased with more dietary protein. Herbert (1992), however, cautioned that the size of the hypopharyngeal glands did not always correspond to the production of glandular secretions adequate for larval growth.

Protein digestion

Pollen protein digestion has been studied in few animals. Pollen protein was efficiently digested by both the larvae of the solitary bee *Chelostoma florisomne* (Dobson and Peng 1997) and adult honey bees (Klungness and Peng 1984), with digestion initiated in the anterior midgut. Rats digested 52–59% of the pollen protein in a diet of pellets of *Eucalyptus* pollens taken from honey bees (Bell et al. 1983), while mice fed *Prosopis velutina* pollen digested 80% of pollen protein (Schmidt et al. 1984). Franchi et al. (1997) studied pollen protein digestion in a test apparatus, including pancreatic enzymes, designed to mimic the human digestive system. They found that after 24 hours, only 48% (*Papaver rhoes*) and 59% (*Corylus avellana*) of pollen protein had been digested. These researchers concluded that the value of pollen as a human dietary supplement was greatly overestimated by simple analyses of pollen chemistry.

Nitrogen assimilation efficiency

Nitrogen assimilation efficiency has been determined for two bee species, *Megachile rotundata* (*M. pacifica*) and *Apis mellifera*. Wightman and Rogers (1978) calculated the nitrogen in the diet, feces, cocoons and mature larvae of *Megachile rotundata* and concluded that the bees assimilated 87.2% of ingested nitrogen. Schmidt and Buchmann (1985) restricted a honey bee colony to its hive in a small flight cage for 4 weeks. They determined the mass of nitrogen consumed and defecated by the entire colony and estimated that the colony assimilated 77–83% of ingested nitrogen. Such

efficient nitrogen assimilation is unparalleled among invertebrates (Wightman and Rogers 1978), a surprising fact given the inherent difficulty of extracting the nitrogen from the pollen grain. Not all bee species, or not all bees on all diets, assimilate nitrogen this efficiently, however. The bee *Osmia lignaria* retained only 35–50% of dietary nitrogen in adult body tissue (Levin and Haydak 1957). This figure excludes nitrogen in cocoons, a very minor nitrogen sink for *Megachile rotundata* (Wightman and Rogers 1978).

Pollen-feeding mammals also efficiently utilize pollen nitrogen. The marsupial *Petaurus breviceps* digested 76% of the nitrogen in its *Eucalyptus* pollen diet and maintained a daily nitrogen balance with the lowest input nitrogen of any marsupial yet recorded (Smith and Green 1987). The Queensland Blossom Bat, *Synconycteris australis*, digested 58–97% of the nitrogen in its laboratory pollen diet (Law 1992a).

Amino acid composition

Most pollens contain all the common amino acids (Solberg and Remedios 1980), but pollen sometimes lacks phenylalanine, tryptophane, hydroxyproline, tyrosine and aminobutyric acid (Johri and Vasil 1961). Tryptophane and phenylalanine are the only essential amino acids sometimes lacking (Lundén 1956). Pollen amino acids that are abundant in the free state also tend to abound in protein (Stanley and Linskens 1974). Solberg and Remedios (1948) reported that the six most common amino acids, aspartic acid, glutamic acid, proline, leucine, lysine, and arginine, comprised 60% of the protein weight of pollen grains. Among free amino acids, proline is often the most abundant and can account for 1–2% of the total weight of pollen grains (Stanley and Linskens 1974).

Although numerous studies have analyzed the amino acid contents of one or a few pollens (e.g. Nielsen et al. 1955, Giliam et al. 1980, McCaughey et al. 1980, Solberg and Remedios 1980, Standifer et al. 1980, Ceausescu and Mosoiu 1981, Rayner and Langridge 1985, Wu

and Murry 1985, Buchmann 1986, Loper and Cohen 1987, Agarwal and Nair 1989, Erhardt and Baker 1990, Clark and Lintas 1992), no study has yet assembled sufficient data to discern taxonomic and ecological trends. Thus, it is not clear if a lack of generalities concerning differences in amino acid content are due to a lack of broad trends or a lack of data. Based on small data sets, Solberg and Remedios (1980) and Lundén (1956) concluded that pollen of wind and bee-pollinated plants contained similar amino acid profiles. McLellan (1977) compared the concentrations of 16 amino acids among seven honey bee-collected pollens. He found significant differences among species for serine, cystine, and histidine, but did not speculate on the causes of those differences.

Honey bees require ten amino acids for maximum development: arginine, histidine, lysine, tryptophane, phenylalanine, methionine, threonine, leucine, isoleucine, and valine (De Groot 1953). Honey bees commonly collect the pollen of dandelion, *Taraxacum officinale* (e.g. Peng et al. 1985), which lacks the essential amino acids tryptophane and phenylalanine (Auclair and Jamieson 1948) and is deficient in arginine (Herbert 1992). Young honey bees fed only dandelion pollen failed to rear brood (Herbert et al. 1970). Dandelion pollen amended with L-tryptophane, L-phenylalanine, and L-arginine promoted full brood rearing (Herbert 1992), but the addition of L-tryptophane and L-phenylalanine alone (Herbert 1992) or L-arginine alone (Loper and Berdel 1980) failed to do so. Adult honey bees also died sooner on a diet of dandelion pollen than on other pollens (Knox et al. 1971).

No extensive studies exist for the amino acid requirements of pollen-feeding animals other than the honey bee. Amino acid requirements are similar for animals as varied as dogs, rats, pigs, humans, and bees (Howell 1974). Thus, it seems likely that most pollen-feeding animals will have similar amino acid requirements. Howell (1974) characterized the amino acids of two species of bat-pollinated plants and found them to be similar to those of bee-

pollinated plants. She speculated that the apparently rich concentration of proline, an important component of collagen, and tyrosine, perhaps a growth stimulant for young bats, in *Agave* spp. and *Carnegiea gigantea* pollen represented biochemical adaptations of the plant to attract their bat pollinators. Such speculation seems premature, however. There is insufficient evidence to distinguish the amino acid profile of any ecological group of plants, such as anemophilous, entomophilous, or chiropterophilous (bat-pollinated) from any other group of plants. Although *Agave* spp. and *Carnegiea gigantea* pollen appear to be rich in tyrosine, they are poorer in proline than various anemophilous plant species (see Solberg and Remedios 1980).

Starch content

Todd and Bretherick (1942) and Roulston and Buchmann (in Press) quantified starch content of 89 plant pollens. Average starch concentration ranged from 0–22% by dry weight. The starchiest pollens were *Typha latifolia* (22.3%), *Zea mays* (20.1%), *Salix* sp. (12.3%) and *Plantago lanceolata* (11.1%) (Roulston and Buchmann in Press), but 41 of the 89 pollens contained less than 1% starch (Roulston and Buchmann, unpublished). Calvino (1952), Baker and Baker (1979, 1983), and Franchi et al. (1996) scored pollen grains categorically as starchy or starchless, based on iodine staining, for ca. 1000 plant species per study. Calvino (1952) and Baker and Baker (1979, 1983) noted that starchy pollen occurred disproportionately often among anemophilous species. Both studies also noted that entomophilous pollens appeared more oily than anemophilous pollens, although neither study quantified lipid content.

Baker and Baker (1979, 1983), and later Grayum (1985), argued that starchy pollen arose as an adaptation to deter pollen theft by non-pollinating insects and/or lipid-rich pollen arose as a plant adaptation to attract and reward pollen-feeding pollinators. Their arguments assume that 1) pollens of plant species pollinated by non pollen-feeding vectors (e.g.

wind, birds, moths) are more likely to be starchy than plant species pollinated by pollen-feeding animals, and 2) pollen-feeding animals prefer non-starchy pollen.

Statistical support for the prevalence of starchy pollen in anemophilous lineages may depend on the statistical techniques applied. Treating every species as an independent sample, Baker and Baker (1979) concluded that the sample of anemophilous species (as well as groups pollinated by birds and lepidopterans) contained a greater proportion of starchy species than the sample of all ca. 1000 taxa analyzed. Roulston and Buchmann (in Press) tested this hypothesis with a sample of 207 plant species arranged into a phylogeny based on published cladograms. Using phylogeny-based statistics, they concluded that starchy pollen was not unusually associated with anemophilous lineages or any lineage in which pollen was not considered a pollinator reward. They concluded that the discrepancy between their results and those of Baker and Baker (1979) resulted from the prevalence of starchy species in three speciose, primarily anemophilous clades that were statistically treated as three independent samples by Roulston and Buchmann but numerous independent samples by Baker and Baker.

No experiments evaluate the preference of pollen-feeding animals for starchy or starchless pollen. Baker and Baker (1979) predicted that pollen-feeding insects would eschew starchy pollen because prior studies reported that honey bees have difficulty digesting starch. More recent work shows that honey bees and other bees can digest starch if they can free it from the amyloplasts and pollen grain (see below). Furthermore, the starchiest pollens known are either often collected by bees (e.g. *Zea mays*) or often used for the rearing of some bee species in the laboratory (e.g. *Typha latifolia*) (Roulston and Buchmann in Press).

Starch digestion

Digestion of pollen starch by bees varies among bee species and/or pollen types. Larvae

of *Chelostoma florisomne* (Dobson and Peng 1997) and *Osmia* spp. (Suárez-Cervera et al. 1994) efficiently digested the pollen starch of their respective host plant pollens, *Ranunculus* spp. and *Cistus* sp. *Osmia cornuta* completely digested the starch of *Euphorbia helioscopia*, but only partially digested the starch of *Ranunculus* sp. (Nepi et al. 1997). Honey bee adults readily digested starch when it was released from the pollen grain but only poorly or not at all in pollen grains that were not degraded during the digestive process (Klungness and Peng 1984). Thus, the presence of starch in the feces of honey bee adults (e.g. Parker 1926) may be due not to an inability to digest starch but rather to an inability to free it from the pollen grain. In *Chelostoma florisomne* larvae, pollen starch was released into the digestive tract (in the median midgut) considerably further along than where protein and lipid digestion began (Dobson and Peng 1997). Differences in starch digestion among pollen types could reflect different storage locations within the pollen grain or different chemical forms of starch. Franchi et al. (1997) found that starch-like short chain polysaccharides dispersed in the cytoplasm of *Papaver rhoes* pollen were much more readily digested than the starch grains located in amyloplasts of *Corylus avellana* pollen when submitted to simulated human digestion.

Lipid content

Lipid content of pollen grains has most often been estimated through ether extraction, which removes 1–20% of the weight of pollen grains (Stanley and Linskens 1974). Ether extracts may include fats, fatty acids, some vitamins, pigments, higher alcohols, waxes, sterols and saturated hydrocarbons (Solberg and Remedios 1980). Solberg and Remedios (1980) concluded that the main ether extractable constituents of 16 hand-collected pollens were linolic, linoleic, and arachidonic acids that occurred in the pollenkitt. Dobson (1988) determined the number and class of pollenkitt lipids for 69 angiosperm species based on benzene then chloro-

form:methanol extraction followed by thin layer chromatography. Dobson (1988) noted that species pollinated by mere nectarivores contained fewer kinds of pollenkitt lipids than species pollinated by bees, presumably because scents in the pollenkitt attract bees seeking pollen. If the role of pollenkitt were primarily one of pollinator attraction, then it might be expected that pollens of plants not pollinated by pollen-feeding organisms would contain less pollenkitt than pollens of plants pollinated by pollen-feeders such as bees, bats, and some flies. An alternate function of pollenkitt is to stick pollen on the body of animal pollinators so that it can be transported to conspecific flowers. Hesse (1979a, b, c) observed that anemophilous pollens tend to have less pollenkitt and less sticky surfaces than closely-related zoophilous species. Thus, it might be expected that zoophilous pollens in general would have more pollenkitt than anemophilous pollens, regardless of whether or not the pollen is consumed by pollinators. If pollenkitt abundance corresponds to sticking pollen grains to animal pollen vectors and, additionally, attracting pollen vectors that also feed on pollen, we might predict that lipid abundance of pollen would follow this approximate pattern: anemophilous < zoophilous < pollen-feeding zoophilous.

This relationship is not apparent in a summary of lipid extractable materials for various plant taxa. Table 2 compiles published data for 62 plant species. The table includes both hand and honey-bee collected samples, because most samples analyzed have come from honey bee samples. The three species richest in ether extractable material are *Taraxacum officinale*, *Juglans nigra*, and *Populus fremontii*. Although it is visited by pollen-collecting honey bees, *Taraxacum officinale* is primarily apomictic (King and Schaal 1990), not requiring any pollination. *Juglans nigra* (Rink et al. 1989) and *Populus fremontii* (Colin and Jones 1980) are wind-pollinated. The three anemophilous Betulaceae species and the two anemophilous Fagaceae analyzed also contain a relatively rich ether extractable fraction. Values for *Pinus* spp., while lower (2–9%),

are higher than some zoophilous species, such as *Eucalyptus* spp., and *Ceanothus* spp. The uncertain precision of values from honey bee-collected samples precludes statistical analyses on all of the published samples, but it seems unlikely that an overall survey of ether extractable fractions would show entomophilous pollens to contain more extracellular lipids than anemophilous pollens. This in no way casts doubt on the well-demonstrated ability of pollen-consuming insects to cue in on pollenkitt lipids (Dobson 1987, 1994) or the role of pollenkitt in attaching pollen to pollinators (Hesse 1979a, b, c); it only casts doubt on the likelihood of either of these specific functions generating an overall association between pollination mode and pollenkitt abundance.

Species analyzed two or more times generally show similar estimates [e.g. *Phoenix dactylifera* (2.2–3.7%), *Trifolium repens* (2.7–3.8%), and *Zea mays* (2.5–6.7%)]. Estimates for *Typha latifolia* (1.4–7.6) and *Populus fremontii* (6.0–17.8) ranged more widely and, as expected, the estimate for hand-collected *Populus fremontii* pollen exceeded the estimate for honey bee collected pollen.

Pollenkitt lipids may comprise a small fraction of total pollen lipids. Ibrahim (1974) found that petroleum ether extracted only 1.6% of *Pinus taeda* pollen contents when whole pollen was placed in the solvent. After the pollen was ground in a mill, thus exposing internal pollen lipids to solvent, petroleum ether extracted 6.1% of its material. Similarly, Evans et al. (1991) found that vortexing the intact pollen of *Brassica napus* in solvent yielded only 27.1% of the total lipids extracted when pollen walls had been fractured prior to vortexing. Estimating the total lipids available to pollen-feeding animals will require many more analyses that rupture the pollen walls before extracting the lipids in solvent.

Lipid digestion

Few insects have been studied for their pollen lipid digestion. Pollen lipids were completely digested by larvae of the solitary, specialist bee

Table 2. Percent ether extractable materials in dry pollen samples. Superscripted numbers indicate source of data. Superscripted letters indicate hand (*H*) or honey bee (*B*) collected samples

	%
Gymnosperms	
Cupressaceae	
<i>Juniperus utahensis</i>	3.2 ^{1,H}
Pinaceae	
<i>Pinus contorta</i>	2.2 ^{2,B}
<i>P. montana</i>	7.1 ^{3,H}
<i>P. radiata</i>	2.0 ^{2,H}
<i>P. sabiniana</i>	3.2 ^{2,H}
Angiosperms	
Dicots	
Aceraceae	
<i>Acer pseudoplatanus</i>	3.6 ^{4,B}
Actinidiaceae	
<i>Actinidia deliciosa</i> ♀	6.9 ^{5,H}
<i>A. deliciosa</i> ♂	1.9 ^{5,H}
Apiaceae	
<i>Lomatium grayei</i>	8.3 ^{1,B}
Asteraceae	
<i>Agoseris glauca</i>	15.4 ^{1,B}
<i>Centaurea solstitialis</i>	7.8 ^{2,B}
<i>Cichorium intybus</i>	9.5 ^{1,B}
<i>Grindelia squarrosa</i>	7.1 ^{1,B}
<i>Silybum marianum</i>	9.0 ^{2,B}
<i>Taraxacum officinale</i>	18.9 ^{1,B}
<i>T. vulgare</i>	16.2 ^{2,B}
Betulaceae	
<i>Alnus glutinosa</i>	9.4 ^{3,H}
<i>A. incana</i>	13.2 ^{3,H}
<i>Corylus avellana</i>	10.0 ^{6,H}
Brassicaceae	
<i>Brassica campestris</i>	10.7 ^{2,B}
<i>B. kaber</i>	14.5 ^{2,B}
<i>B. nigra</i>	9.9 ^{2,B}
Brassicaceae sp.	7.8 ^{4,B}
Chenopodiaceae	
<i>Salsola kali</i>	7.1 ^{1,B}
Clusiaceae	
<i>Hypericum perforatum</i>	3.2 ^{2,B}
Ericaceae sp.	1.7 ^{4,B}
Fabaceae	
<i>Medicago sativa</i>	8.5 ^{1,B}
<i>Melilotus</i> sp.	8.5 ^{1,B}
<i>Robinia pseudoacacia</i>	12.1 ^{1,B}
<i>Trifolium alexandrinum</i>	6.1 ^{7,B}
<i>T. pratense</i>	14.4 ^{1,B}
<i>T. repens</i>	2.7 ^{4,B} , 3.8 ^{2,B}
<i>Trifolium</i> sp.	3.7 ^{2,B}
<i>Vicia faba</i>	4.0 ^{7,B}
<i>Fabaceae</i> sp.	3.2 ^{2,B}
Fagaceae	
<i>Fagus sylvatica</i>	11.8 ^{4,B}
<i>Quercus kelloggii</i>	7.4 ^{2,H}

Table 2 (continued)

	%
Juglandaceae	
<i>Juglans nigra</i>	18.3 ^{2,H}
Myrtaceae	
<i>Eucalyptus calophylla</i>	1.1 ^{8,B}
<i>E. globulus</i>	1.5 ^{2,B}
<i>E. marginata</i>	0.8 ^{8,B}
<i>Eucalyptus</i> spp.	2.3 ^{7,B}
Oleaceae	
<i>Olea europaea</i>	5.2 ^{2,B}
Onagraceae	
<i>Epilobium angustifolium</i>	2.0 ^{4,B}
Polygonaceae	
<i>Polygonum</i> sp.	1.5 ^{1,B}
Portulacaceae	
<i>Calandrinia ciliata</i>	6.2 ^{2,B}
Ranunculaceae	
<i>Ranunculus arvensis</i>	5.7 ^{1,B}
<i>Ranunculus</i> sp.	2.3 ^{4,B}
Rhamnaceae	
<i>Ceanothus crassifolius</i>	1.3 ^{2,B}
<i>C. integrerrimus</i>	1.0 ^{2,B}
Rosaceae	
<i>Chamaebatia foliolosa</i>	5.0 ^{2,B}
<i>Malus pumila</i>	10.4 ^{1,B}
<i>Prunus communis</i>	3.5 ^{2,B}
<i>P. serotina</i>	10.7 ^{1,B}
<i>P. persica</i>	3.0 ^{2,B}
Salicaceae	
<i>Populus fremontii</i>	6.0 ^{1,B} , 17.8 ^{1,H}
<i>P. nigra italicica</i>	8.9 ^{1,H}
<i>Salix nigra</i>	4.7 ^{2,B}
<i>Salix</i> sp.	6.1 ^{2,B}
Monocots	
Arecaceae	
<i>Phoenix dactylifera</i>	2.2 ^{7,B} , 3.7 ^{2,B}
Asparagaceae	
<i>Asparagus officinalis</i>	4.5 ^{2,B}
Poaceae	
<i>Cynodon dactylon</i>	2.7 ^{2,B}
<i>Zea mays</i>	2.5 ^{1,B} , 3.9 ⁹ , 6.7 ^{7,B} , 1.8 ^{3,H} , 5 ^{3,H} , 3.9 ^{2,H}
Typhaceae	
<i>Typha latifolia</i>	1.4 ^{2,H} , 7.6 ⁹

¹ Standifer (1966)² Todd and Bretherick (1942) (%lipid - %water)³ Nielsen (1955)⁴ McLellan (1977)⁵ Clark and Lintas (1992)⁶ Sosa and Sosa-Bourdouil, in Barbier (1970)⁷ Ibrahim (1974)⁸ Bell et al. 1983 (1983)⁹ Gunasekaran and Andersen 1973, in Stanley and Linskens (1974)

Chelostoma florisomne (Dobson and Peng 1997), but incompletely digested by larval *Osmia* spp. (Suárez-Cervera et al. 1994) and adult honey bees (Peng et al. 1985).

Sterol content

Unlike vertebrates, which can synthesize sterols, insects and other arthropods require dietary sterols for the production of certain hormones, such as ecdysone (Blum 1985). For exclusively herbivorous pollen-feeding arthropods, such as most bee species, dietary sterols come from pollen. Most pollens contain sterols, generally < 1% by dry weight (Stanley and Linskens 1974). The particular sterols determined from the few pollens extensively analyzed are described by Barbier (1970) and Stanley and Linskens (1974). Too few pollens have been analyzed for sterols to compare overall taxonomic or ecological associations with sterol abundance. The most important sterols for honey bees appear to be cholesterol and 24-methylene cholesterol (Svoboda et al. 1980). For details of sterol chemistry in honey bees, see Svoboda et al. (1980, 1982, 1987) and Herbert (1992). Sterols may also serve as pollinator attractants. Hügel (1962) reported that honey bees were attracted to pollen sterols, but Herbert (1992) reported that adding sterols to artificial diet did not increase the diet's attractiveness to honey bees.

Vitamin content

Pollens are rich in water soluble and poor in fat soluble vitamins. Barbier (1970) compiled the known concentrations of vitamins ($\mu\text{g/g}$) in pollens: thiamine (1.4–10.8); riboflavin (5.6–19.2); nicotinic acid (40.7–210); pyridoxine (3.1–9); pantothenic acid (4.2–51); biotin (0.25–0.69); folic acid (3.4–6.8); inositol (3–30 mg/g); ascorbic acid (152–640); vitamin A (0); vitamin D (0.2–0.6); vitamin E (0–0.32); vitamin K (0). Nielsen et al. (1955) reported that the pollen vitamin concentration of the gymnosperm *Pinus montana* generally equalled that of the angiosperms *Zea mays*, *Alnus incana*,

and *Alnus glutinosa*. Herbert (1992) reviewed studies of the dietary vitamin requirements of honey bees. Although synthetic vitamin-deficient diets have been shown to hinder hypopharyngeal gland development and brood rearing, we do not know if differences in vitamin concentration of natural pollens influence development of any pollen-feeding animal.

Caloric value of pollen

The caloric value of pollen, based on bomb calorimetry, ranges from 3635 cal/g for *Broussonetia papyrifera* (Moraceae) to 6750 cal/g for *Ambrosia chamissonis* (Asteraceae) for the set of 100 species analyzed from hand-collected samples (Colin and Jones 1980, Petanidou and Vokou 1990, Shah 1997; excluding honey bee-collected pollens analyzed by Loper and Cohen 1982). Petanidou and Vokou (1990) stated that entomophilous pollens were more calorie-rich than anemophilous pollens, a conclusion not shared by Colin and Jones (1980) or Shah (1997). Petanidou and Vokou (1990) hypothesized that calorie-rich pollen was a plant adaptation to reward insect pollinators. Importantly, four of Petanidou and Vokou's nine most calorie-rich pollens were entomophilous pollens of the Asteraceae, whereas five of the six most calorie-rich pollens in Colin and Jones (1980) data set were anemophilous pollens from the Asteraceae. This clearly underscores the critical importance of phylogeny and the need to sample and compare closely-related taxa that differ in pollination mode. The three studies of caloric content of hand-collected pollen include four plant families from which researchers sampled both anemophilous and entomophilous species. For two of those families, the average caloric content of anemophilous pollen is greater (Asteraceae and Plantaginaceae), whereas for the remaining two families the average entomophilous value is greater (Salicaceae and Anacardiaceae).

Bomb calorimetry values of pollen grains do not correspond simply to digestible calories because they include calories attributable to

poorly digestible portions of the pollen grain, such as the pollen wall. The percent of calories could vary substantially due to differences in pollen surface-to-volume ratios of different sized and shaped grains (Simpson and Neff 1983). We know of no previous calculations, however, that associate pollen wall material with pollen grain size. Cellulose (1–10%) and sporopollenin (4–22%), two main cell wall components, account for a total of 7–23% of the weight of pollen grains (Kwiatkowski and Lubliner-Mianowska 1957). Shah (1997) took a novel approach to subtracting the caloric value of indigestible material from his estimates of caloric values of pollen grains. He acetolyzed the pollen grains of 18 angiosperms and compared the bomb calorimetry values of acetolyzed and unacetolyzed pollen for each species. Because acetolysis removes all of the pollen grain except the sporopollenin-rich exine, he was able to estimate the caloric value of sporopollenin-free pollen. Based on Shah's data, we calculate that the pollen calories attributable to sporopollenin ranged from 4–42% of the total calories per unit weight (whole pollen caloric value correlated with sporopollenin-free pollen, $r = 0.8$). Variation in percent calories due to sporopollenin was not associated with pollen grain diameter ($r^2 = 0.005$, $n = 17$, after removing one outlier), in contrast to Simpson and Neff's (1983) prediction that smaller grains would contain a greater proportion of cell wall material. Exine thickness has been shown to increase with pollen diameter (Lee 1978), which could at least partially negate the effect of decreased surface area of larger grains in terms of digestible calories per unit weight. Exine thickness does not always increase with pollen volume. Grass pollens are often very large but possess very thin exines.

Some adaptive hypotheses have been invoked to predict that entomophilous plants reward or attract their pollinators with calorie-rich pollen (Petanidou and Vokou 1990) or lipid-rich pollens (as estimated by ether extraction, see section on Lipids). Both arguments fail to account for the most calorie-rich

or lipid-rich pollens being anemophilous. These two hypotheses may be closely related, however. Not only would a thicker layer of pollenkitt increase a pollen grains' ether-extractable fraction, it would also increase its caloric value. The three plant families producing the most calorie-rich pollens are Asteraceae, Agavaceae, and Juglandaceae. Pollen of the Asteraceae and Juglandaceae, plus the Salicaceae, also yield the most ether-extractable material. The pollen of very few individual species has been analyzed for both caloric value and ether-extractable fraction. Various plant families, however, contain species analyzed for one or the other of the two traits. We have taken these published species values, averaged them across each of 17 plant families, and plotted the average number of calories per family against the average percent ether-extractable fraction (Fig. 1). Many of the species included in the ether-extractable portion have been analyzed only from pollen loads of honey bees. They were included because of the paucity of analyses using hand-collected pollens. Arbitrarily increasing the estimates from honey bee-collected samples to account for added sugar by 20% would lower the r^2 to 0.59, whereas increasing the estimate by 40% would increase the r^2 to 0.80. Despite this imprecision, it is apparent that pollen calorie content is strongly associated with the amount of pollenkitt. An unequivocal evolutionary explanation has yet to materialize for different quantities of pollenkitt lipids being produced on pollen of different plant species, although these lipids can serve to attract pollinators (Dobson 1988), feed pollinators (Petanidou and Vokou 1990), stick pollen to pollinator bodies (Hesse 1979a, b, c), or perform various other functions (see Pacini 1997).

Water content

Pollen generally contains less than 20% water, but some grasses may contain 50% or more water at the time of dispersal (Stanley and Linskens 1974, Pacini, this volume). Variation in water content among pollens may offset or

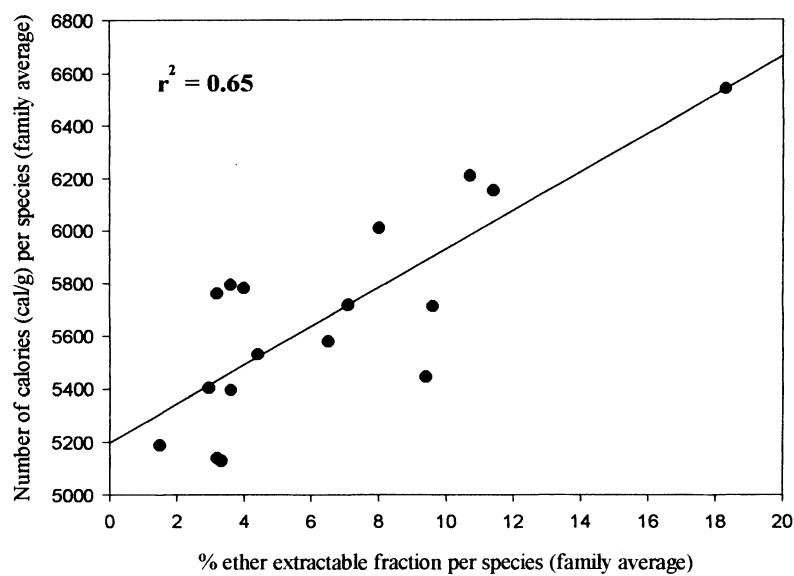


Fig. 1. Caloric value of pollen (cal/g) per plant family plotted against %ether extractable material per plant family. $p < 0.001$

accentuate differences in nutrient content that have been assayed on a dry weight basis. Unfortunately, water content has been estimated for very few pollens, and presently we do not know how it varies with pollen nutrient content.

Toxic pollen?

Various authors have cited studies that reputedly demonstrate that certain pollens are toxic to bees, particularly honey bees (reviewed in Wcislo and Cane, 1996). Suspected pollens come from species of *Aconitum*, *Aesculus*, *Digitalis*, *Ranunculus* (O'Neal and Waller 1984), *Andromeda*, *Astragalus*, *Corynocarpus*, *Hyoscyamus*, *Polygonum*, *Rhododendron*, *Scolypoda*, *Tilia* and *Veratrum* (Stanley and Linskens 1974). In some cases, although subsequent authors cite the toxin source as being pollen, the original studies report extracting whole stamens or even florets, and not just pollen. Other studies [e.g. *Astragalus* (Vansell 1934); *Veratrum* (Vansell 1933)] failed to distinguish the effect of nectar from the effect of pollen in poisoning foragers or brood. Toxic nectars are well known among plants and it has been suggested that they serve to promote pollinator constancy by reducing the visitation of some animal groups, such as lepidopterans,

thus providing all of the floral reward for other groups, such as bees (reviewed in Rhoades and Bergdahl 1981). Hitchcock (1959) shook pollen from the flowers of *Zigadenus venenosus* into sugar water and killed 89% of the bees that fed on it within 16 hours. Thus, it appeared that the toxins, probably alkaloids (Tepedino 1981), were sufficiently abundant in pollen to have a toxic effect. Theoretically, toxins in pollen could guard against pollen foraging by non-pollinators. *Zigadenus venenosus*, however, is apparently bee-pollinated (Moldenke 1976). Tepedino (1989) reported that three bee species of the genus *Dialictus* as well as the putative *Zigadenus* specialist *Andrena astragali*, collected pollen from *Zigadenus nuttallii*. Successful development of any of these bees on *Zigadenus* pollen, however, has not yet been observed. No specialist bee species (reviewed in Wcislo and Cane 1996) have been shown to digest pollens that cannot be digested by generalist bee species.

Toxic substances in some pollens include mannose sugars (Crane 1977, 1978), various alkaloids (Detzel and Wink 1993), and polyphenolics (Carisey and Bauce 1997). Defensive compounds in pollen, when present, are often similar in composition but more dilute than those present in leaves of the same species. Carisey and Bauce (1997) found that concen-

trations of total phenolics and tannins were similar between pollen and foliage of *Abies balsamea*, but that monoterpenes were greatly diluted in pollen. Detzel and Wink (1993) surveyed pollen alkaloid content of *Atropa belladonna*, *Lupinus polyphyllus*, *Brugmansia aurea*, and *Nicotiana tabacum*. All contained alkaloids, but in greatly diminished quantities relative to leaf tissue (*Atropa belladonna* was not analyzed for leaf alkaloids). Nectar contained lesser quantities of alkaloids than pollen in two of the three species surveyed for both. Although Detzel and Wink (1993) carried out alkaloid repellency and toxicity studies with honey bees, it is unclear whether or not the alkaloid concentrations present in all species except *Atropa belladonna* (which contained greater quantities of pollen alkaloids) would repel or poison foraging honey bees. Roulston and Cane (submitted) fed the pollen of *Brugmansia candida* to laboratory colonies of the generalist sweat bee *Lasioglossum zephyrum*. The bees collected and consumed the pollen readily. Their progeny attained as large or larger average body size when fed just *B. candida* pollen than on any of seven other pollens, reflecting this pollen's rich protein content (Roulston et al., in press).

Suggestions for future research

The literature on pollen digestion contains more variables (species examined and pollen characteristics) than equations (experimental observations), which makes it difficult to discern generalities from idiosyncracies. Two specific pollen characteristics have been hypothesized to influence digestibility: pollen wall (both entine and exine) thickness and presence and number of germination pores. Feeding trials with one organism using pollens that differ in these characteristics will help move the study of pollen digestion from a field of opportunistic observations to one of hypothesis testing.

The peculiar specialized "pollen tooth" found on the mandibles of some beetles may represent a rare morphological adaptation associated with pollen mastication. However,

this putative mechanical function has not been satisfactorily demonstrated, so we can not yet conclude that this morphological correlation with pollen-feeding has indeed arisen in order to overcome the resistant pollen wall.

Pollen pellets taken from foraging honey bees are frequently used for chemical analyses of pollen. However, because nectar sugars can add substantial weight to these pellets, they affect all calculations for concentrations of pollen constituents. We lack a reliable laboratory method for removing these nectar sugars from pollen pellets without inadvertently extracting pollen constituents. No study has ever specifically determined the ratios of pollen to nectar-derived sugars in these pellets. We know neither the variability of this ratio, nor what factors control the proportion. Understanding such factors, which are mostly behavioral, will provide insights into the reliability of using these pollen pellets collected by honey bees to quantify pollen constituents. Until these vexing analytical problems are resolved, we must cautiously interpret studies of pollen chemistry that employ as their starting material either harvested whole stamens of flowers or pollen pellets taken from foraging social bees.

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The collection of pollen by bees

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Abstract. Bees require pollen for their reproduction and pollen comprises the basic larval food for bees. Most bees acquire pollen passively during flower visitation, but female bees may also collect pollen actively with the aid of various structural and behavioral adaptations. Most bees have evolved adaptations to concentrate pollen into discrete loads and transport pollen back to their nests. The various structural and behavioral adaptations of female bees for acquiring and transporting pollen are the basis of this review.

Key words: Bees, pollen collection, pollen transport, pollen grooming, pollen removal.

Twenty-years ago I reviewed the structural, behavioral, and physiological adaptations of bees for collecting pollen for a symposium held at the Missouri Botanic Garden (Thorp 1979b). The invitation to submit a paper to this volume gives me the opportunity to revisit recent literature and update information in the area. Structures and behaviors for collection and transport of oils from plants were treated by Roberts and Vallespir (1978) and Thorp (1979b), and updated by Neff and Simpson (1981) and reviewed by Buchmann (1987) and the evolution of host plant specificity for pollen collection, oligolecty, was recently treated by Wcislo and Cane (1996) and Müller (1996b). These topics will not be treated further here. I will address the topic from the

viewpoint of the bee, recognizing that this considers only part of the complex interactions and adaptations between flowers and their guilds of visitors. As has been pointed out by other authors, flowers often develop counter-measures to limit the amount of pollen that can be obtained by bees on a single visit so as to enhance the chances that their pollen will reach stigmas of flowers of their species rather than disappear from the system to become food for bee larvae (Westerkamp 1996, 1997a, b), especially through development of different methods of packaging or dispensing (Harder and Thomson 1989). The morphological terminology and classification of bees used in this review follow those of Michener et al. (1994).

Among the Apoidea the apiformes (bees) and spheciformes (wasps) share a common ancestry (Michener et al. 1994). The ancestral habit of provisioning nests with animal food such as insects and spiders is maintained in the spheciform wasps. Bees have diverged from their wasp-like ancestor to use pollen as the primary food for their brood. Along with this change in food habit a number of structural and behavioral changes have appeared in the bee line. Adult bees have branched body hairs not found among the wasps. Even male bees are often hairy and accumulate considerable pollen on their bodies while foraging for nectar. Thus they may play significant roles

in mess-and-soil type pollination. Although some female bees carry pollen internally in their alimentary canals, most have special external devices for transporting pollen gleaned from flowers back to their brood nests. They also exhibit modified structures and behaviors for grooming pollen from their bodies and loading it into their pollen transport structures. In addition, a few bees possess specific structures for acquiring pollen from specific flowers. Some have hooked hairs on mouthparts or forelegs to facilitate collecting pollen from flowers with hidden anthers. Others have additional clusters of hairs, usually on the head, which aid in accumulating pollen from flowers, especially those with nototribic pollen presentation. Some bees exhibit special behavioral movements to enhance the uptake of pollen, including vibration of flowers, especially in plants with poricidal anther openings.

Structural adaptations of bees for pollen transport

The external structures involved in pollen transport from flowers to the nest come in a variety of forms. In most bees, these consist of brushes of hairs (scopae) on the hind legs (Thorp 1979b). In the Megachilidae (including the Fidelinae), the scopa is confined to the venter of the metasoma (Whitehead 1984). Bees with hind leg scopae may also have supplemental pollen bearing hairs on the metasomal venter (Thorp 1979b).

The density and plumosity of scopal hairs are related to the size and structure of the pollen grains female bees transport and whether or not fluids are added to the pollen loads (Thorp 1979b). The use of nectar to moisten pollen loads as found in bees with sparse scopae such as, Panurginae and Mellitidae, and bees with hind tibial corbiculae, Apidae, permits considerable flexibility in sizes of pollen grains that may be collected (Thorp 1979b). Houston (1989) describes the sparse scopae of females of *Leioproctus* species that are oligoleges of *Conospermum* (Proteaceae).

He relates this to the rather large pollen grains, 80–90 μ diameter, and suggests that full loads extending beyond the scopal hairs "must be bound by some adhesive." Other bees that "top off" their pollen loads moistening them with nectar near the end of their foraging trips to extend the size of the load beyond the length of the scopal hairs include *Stenotritus* and *Ctenocolletes* (Stenotritidae) (Houston and Thorp 1984, Houston 1984).

In the Apidae (*sensu strictu*), the hind tibia is modified to a flat plate surrounded by long hairs, the corbiculum, where pollen is stored as a moist lump (Michener et al. 1978). Corbiculae structures may be found on other body parts of other bees, such as on the lateral propodeum in Andrenidae, and on the lower side of the hind femur in families including the Colletidae, Oxaeidae, Andrenidae, and Halictidae (Thorp 1979b, Michener 1999). Michener (1999) further points out the corbicula-like structures on the outer apical area of the hind tibiae in the tribe Eucerini and describes the distinct hind tibial corbiculae of *Canephorula apiformis*. Westerkamp (1996) suggests that corbiculae are also formed within the metasomal scopa in some Megachilidae (e.g. *Megachile willoughbiella*). In non-Apidae corbiculae, pollen is not moistened, but packed dry (Michener 1999).

The structures involved in pollen grooming and pollen transport from flowers to the nest are presented in tabular form in Roberts and Vallespir (1978) and Thorp (1979b). Some emendations and additions to these lists need to be made based on the works of several authors. Zavortink (1972) describes scopal hairs on the first two metasomal sterna of *Megandrena (Erythrändrena) mentzeliae* in addition to the hind leg scopae and propodeal corbiculae typical for Andrenidae. Neff (1984) describes specialized setae on the venter of *Eremapis*, an exomalopsine anthophorid, and provides evidence that these serve not only to collect pollen, but as a metasomal scopa in addition to the hind leg scopae to transport pollen to the nest. Eickwort et al. (1986) point out that Rophitinae (as Dufoureinae) do not

carry pollen extensively on the hind trochanter or metasomal sterna as is true for other Halictidae. Winston (1979) did not find a galeal comb in *Ceratina* and concludes that all long-tongued bees (Ctenoplectridae, Megachilidae, Anthophoridae, Apidae) lack such combs. In addition, Winston (1979) found stipetal combs in some anthidiine megachilids and in *Ctenoplectra*.

Structural adaptations of bees for pollen collection

Hooked or modified hairs on the mouthparts or forelegs of female bees that extract pollen from tubular flowers with hidden anthers, such as in Boraginaceae and Verbenaceae, have long been known (Shinn 1967, Thorp 1979b). More recent examples include: *Osmia sculleni* (Megachilidae) on *Cryptantha* (Boraginaceae) in Utah (Parker and Tepedino 1982), *Leioproctus* (*Leioproctus*) *capito* species-group (Colletidae) on *Eremophila* (Myoporaceae) (Houston 1990), *Leioproctus macmillani* (Colletidae) on *Astroloma* (Epacridaceae) (Houston 1991a), *Melissodes apicata* (Anthophoridae) on *Pontederia* (Pontederiaceae) in eastern Canada (Harder and Barrett 1993). In addition, Müller (1995) found 13 species of bees in central Europe (Colletidae, Andrenidae, Megachilidae, Anthophoridae) with similar adaptations, and most of these bees collect pollen from Boraginaceae and Primulaceae. Houston (1989) suggests that the "simple erect setae on the prementum and stipes" of females of the *Leioproctus conospermi* species-group may aid in extracting and holding extra pollen. Thus, they would function as the hooked mouthpart hairs of other bees.

Not included by Thorp (1979b) were the modified hairs on the heads and venters of various female bees for the extraction of pollen from their host flowers. In the Old World, Müller (1996a) describes the convergence between the specially modified clypeal hairs of the masarid (honey) wasp, *Celonites abbreviatus*, and similarly modified facial hairs of 13 species of bees in the families Halictidae,

Megachilidae, and Anthophoridae found in central Europe. In the halictid bees of the genus *Rophites*, the specially modified hairs are on the frons between the antennal bases and the ocelli (Müller 1995). In most of these Old World bees, the special hairs are used to remove pollen from nototribic flowers of Lamiaceae and Scrophulariaceae.

In North America, LaBerge (1970) records similarly modified clypeal hairs in a new genus of eucerine bees he described, *Pectinapis*, including one species with a row of stiff flattened setae (the pectin) just above the clypeus. He suggests that the modified hairs are for removal of pollen, although no floral records for these bees were known. Subsequently LaBerge (1989a) reviewed *Pectinapis*, and included several records of females having been collected on nototribic flowers of the Lamiaceae, mostly *Salvia* with one record on *Monarda*. Other North American bees with similar modifications for pollen extraction include: Andrenidae: *Andrena* (*Simandrena*) *pensilis*, *A. (S.) orthorcarpi* (LaBerge 1989b), *Panurginus atriceps* (Thorp personal observation, see below); Megachilidae: *Trachusa* (*Ullanthidium*) spp. (Thorp and Brooks 1994); Anthophoridae: *Anthophora* (*Mystacanthophora*) spp., *Habropoda* spp. (2), and *Xenoglossodes* (Brooks 1988) and *Deltoptila* spp. (LaBerge 1989a). According to Brooks (1988), other possibilities include: "an undescribed subgenus of South American *Megachile* (Moure pers. comm.)" and "an undescribed genus of Eucerini related to *Eucera* in Africa." Floral hosts are not well documented for many of these New World bees. However, the facial hair adaptations seem best suited for removal of pollen from nototribic flowers as has been found in the Old World. Obviously these types of pollen gleaning structures have arisen independently a number of times in the Apoidea in both the Old and New World.

Another structural adaptation was described by Snelling and Stage (1995). They found a "brush of long, curled hairs on the lower genae" of female *Xeralictus* (Halictidae, Rophitinae). This brush on the underside of

the head accumulates pollen as do the forelegs which scrape audibly while females forage deep in flowers of *Mentzelia* (Snelling and Stage 1995).

Passive pollen accumulation

A distinction has been drawn between passive (incidental) and active modes of pollen accumulation on bees visiting flowers (Doull 1970, Westerkamp 1996, and Bernhardt 1996). Passive accumulation of pollen on the external surfaces of bees during visits to flowers to obtain nectar is considered the more primitive method of pollen extraction from flowers. Perhaps the most extreme mode of passive accumulation of pollen is on bees that visit plants where scores of pollen grains are packaged in pollinia such as orchids or milkweeds. These structures may be transported by bees, but are not known to be collected by them for use in their reproduction. Pollen grains may be clumped by the formation of tetrads or polyads (e.g. Ericaceae, Onagraceae, Fabaceae). Tetrads or single grains also may be tied together with viscin threads (e.g. Onagraceae, Ericaceae) (Raven 1979; Hesse 1980, 1981; Waha 1984). Bees that specialize in collection and transportation of loads of webby large pollen grains of Onagraceae often show modifications of their scopae, especially simplification and sparsity of scopal hairs (Thorp 1979b, Pasteels and Pasteels 1979, and Gimenes 1991).

An autapomorphic character that defines bees among the aculeate Hymenoptera, namely the possession of branched hairs, is an adaptation that assists in the passive acquisition of pollen on the body surfaces of bees. From this position, some pollen grains may function in the reproduction of plants via pollen transfer (pollination), while others may be groomed and packed by females of many bees into various pollen transport devices to be carried back to bee nests and used as bee food. Females of the colletid subfamilies Hyleinae and Euryglossinae transport pollen internally (Thorp 1979b). They are relatively hairless.

Cleptoparasitic [cuckoo] bees, which lay their eggs in nests of pollen collecting bees, and rely on the pollen provisions and young of their host bees as food sources for their young have modified body hairs. Many (Nomadini) are relatively hairless and the others (Epeolini) have appressed scale-like hairs. These adaptations reduce the amount of pollen accumulated while foraging for nectar, and would reduce the time required to groom and remove pollen from their bodies.

Passive acquisition of pollen may be enhanced by electrostatic attraction of differently charged hairy bee bodies and pollen (Erickson and Buchmann 1983). This topic is reviewed in this volume by Vaknin et al. (2000). It would be interesting to determine whether the cleptoparasitic bees mentioned above and other relatively hairless bees (e.g. hylaeine colletids) actually acquire less pollen via electrostatics than do their hairy pollen-collecting relatives.

Bees may be quite efficient at grooming and packing pollen into transport structures. Packed pollen is no longer available to the pollination process, especially on bees that have corbiculae or that transport pollen in moist loads on hind leg scopae. Bees that have ventral scopae, especially Megachilidae, and that pack their pollen loads dry, may still have much pollen available for transfer to stigmas. Considerable recent research over the past 10 years has focused on measuring differences in ratios of pollen removal from anthers and pollen deposition on stigmas by flower visitors of many plants to assess the relative pollination efficiency in diverse guilds of potential pollinators (e.g. Harder and Thomson 1989). This "hot" topic is beyond the scope of the present review, but see Harder and Wilson (1997) for a review of the theoretical aspects.

Some pollen obtained passively never makes it to floral stigmas nor back to bee nests. Bees, especially nectar foraging honey bees, sometimes become copiously covered with pollen at some flowers, such as sunflower and cotton (Parker 1981, Vaissiere and Vinson 1994). Rather than packing this pollen, the bees often groom and discard it. Neff and

Simpson (1997) describe this discarding behavior in nectar foragers of *Andrena rudbeckiae*. Only pollen deposited out of the reach of a bee's grooming structures, i.e. in "safe sites," is likely to reach stigmas of subsequently visited flowers. Nototribic flowers that place pollen in safe sites along the bee's dorsal midline where they cannot easily groom all the pollen, and where pollen is in an optimal position for deposition on stigmas. The grooming behaviors of euglossine bees is described in detail by Kimsey (1984) who illustrates the large areas of the body that are not cleaned and thus serve as safe sites for pollen and pollinaria, as well as for phoretic mites and parasites.

Active pollen removal from flowers

Active pollen removal involves behavioral modifications that cause the release of pollen from flowers. Many of these behaviors rely on standard morphological equipment of bees such as mandibles, foretarsal brushes, flight musculature, and some are performed in concert with the structural adaptations for pollen collection mentioned above.

Some bees actively collect pollen from anthers of flowers designed to transfer pollen to passive pollen-collecting visitors. Such bees may be greatly mismatched in size with some flowers they visit for pollen. Furthermore, pollen and nectar collection (if nectar is present) are very separate activities at these flowers. For example, small females of *Lasioglossum* (Halictidae) actively collect pollen from the exserted anthers of *Keckiella cordifolia* (Scrophulariaceae) on Santa Cruz Island, California (personal observation). The long, tubular, red flowers of *Keckiella* with exserted stamens and styles are typical of flowers adapted to hummingbirds as the primary vectors of pollen. Barthell and Knops (1997) found carpenter bees actively collecting the webby pollen pollen of *Oenothera elata*. The flowers open before sunset, remain open until mid morning, and were previously thought to be solely hawkmoth-pollinated. These authors

found carpenter bees visiting at dusk and dawn, grasping anthers with their legs, transferring pollen to their scopae and depositing pollen on 56–70% of stigmas monitored. Small generalist halictines may climb out on stamens of large flowers adapted for hawkmoth pollination and scrape pollen directly from anthers that are remote from stigmas, thereby acting as pollen thieves (e.g. *Evylaeus* sp. on *Datura* on Santa Cruz Island, California, Thorp personal observation). Even specialist (oligolectic) bees may exhibit pollen thieving behavior (e.g. *Perdita wootonae* on *Mentzelia decapetala*, Michener 1979).

Sonication behavior. Over the past 20 years, one of the most discussed behaviors for active pollen collection is vibratile or buzz pollination behavior as described by Buchmann (1983). This arises from a modification of bee flight in which the indirect flight muscles of the thorax are activated in a shivering mode while the wings remain disengaged and reflexed over the back. This behavior occurs primarily when bees alight on flowers with poricidal anthers, such as *Solanum* (Solanaceae). The sound produced during a buzzing bout is usually louder and of higher pitch than the sound of the bee in flight and may be audible over several meters.

A great diversity of plant families and genera have poricidal anthers, and most are presumably buzz pollinated (Buchmann 1983). Gottsberger and Silberbauer-Gottsberger (1988) review case histories of buzz pollination in Cassiinae (Fabaceae) and Macior (1982, 1995) presents an overview of his many studies on buzz pollination in the genus *Pedicularis* (Scrophulariaceae). Proença (1992) adds Myrtaceae to the list of buzz pollinated plants based on her observations in Brazil. She found that bees only buzzed those species that had prolonged hypanthial cups and suggested that these cups provided important purchase sites for the bees to hang onto while sonicating. She extrapolates from her findings that about half the tribe Mertoideae may be buzz pollinated (about 1500 species) and supports the contention of Renner (1989) that the origin of buzz

pollination may have been in the lower mid-Cretaceous.

Diverse bee taxa are involved in the buzz pollination syndrome, especially Colletidae, Oxaeidae, Halictidae, Anthophoridae, and Apidae (except *Apis*). Houston and Thorp (1984) also observed sonication in Stenotritidae. Sonation is uncommon in Andrenidae and Melittidae, and especially rare in Megachilidae (Neff and Simpson 1988). An exception is *Megachile mendica* which was observed to sonicate flowers of *Chamaecrista fasciculata* (Fabaceae) by Neff and Simpson (1988). After intense search, they noted only a faint buzzing sound weaker than the sound of flight activity. They noted that *Megachile willoughbiella* had been previously reported to exhibit behavior suggestive of buzz pollination. Buzzing behavior has since been confirmed for *M. willoughbiella* on *Rhinanthus* by Müller et al. (1997).

A novel combined behavior method termed "buzz milking" is described by Cane and Buchmann (1989) for *Protandrena* on *Solanum elaeagnifolium*. A female bee curls around the base of the anther and buzzes as she works toward the tip, then curls over the tip with the mid-venter appressed against the apical pores. She continues to buzz with the sound and pitch increasing as pollen is ejected. She then leans back holding onto the anther with her hind legs and grooms the pollen packing it onto the hind legs while adding nectar to moisten the load.

Vibrations produced by bumble bees and measured by an accelerometer showed differences within species, namely in *Bombus terrestris* foraging on kiwifruit (*Actinidia*) or on comfrey (*Symphytum*) and between species, namely *B. terrestris* and *B. hortorum* on comfrey (King 1993). When buzzing younger flowers of kiwifruit, less pollen was released and the presence of tapetal fluid caused the pollen to clump (King and Ferguson 1994). As flowers aged, the anthers dehisced further, pollen became drier, and larger amounts of pollen were released. The authors suggest that the dry pollen of older flowers would be more difficult to groom and pack, and that foragers that vibrate would have an advantage in being

able to release more pollen from young flowers than foragers that do not. The ability to release dry pollen from older kiwifruit without sonication behavior may explain the higher visitation by honey bees to three- and four-day old pistillate flowers observed in one data set by Goodwin and Steven (1993).

Harder and Barclay (1994) examined the efficiency of pollen removal from *Dodecatheon* (Primulaceae) anthers by bumble bees. They found that the anthers of young flowers required higher vibration frequencies than bumble bees could produce so that not all pollen is released. As the flower aged however, a higher proportion of pollen from virgin flowers was removed by bumble bees. Thus, the anthers respond as dispensers limiting the amount of pollen released initially, but releasing more pollen when visitation frequency is low.

Buchmann and Cane (1989) showed experimentally that bees were able to assess the amount of pollen released during sonication bouts on flowers of *Solanum*. They suggest that a positive feedback of pollen amount released is used by both generalist and specialist (oligolectic) bees to adjust their handling time, numbers of buzzes, and groomings per flower. Harder (1990) found similar results with bumble bees on *Dodecatheon* and *Lupinus*, suggesting that bumble bees can determine and respond to differences in pollen availability.

During field studies in Western Australia in October 1981, I was impressed with the diversity of buzz pollinated plants. In addition to *Solanum*, I found bees (especially female *Amegilla*) buzzing *Cassia* (Fabaceae), *Hibbertia* (Dilleniaceae), *Keraudrenia* (Sterculiaceae), and *Tetratheca* (Tremandraceae). Additions to this list include: *Amegilla* vibrating *Trichodesma* (Boraginaceae) (Houston 1991b), and *Stenotritus* sonicating *Cheiranthera* (Pittosporaceae) (Houston and Thorp 1984). The diversity of visitors to *Solanum* in Australia and the types of behavior they use to collect pollen are discussed by Anderson and Symon (1988). Of the more common visitors, *Amegilla* (Anthophoridae) and *Nomia* (Halictidae) used vibration to release pollen. Bernhart (1984, 1986)

describes the buzz pollination of two species of *Hibbertia* primarily by halictid bees and suggests that comparative pollination studies within the "primitive" family Dilleniaceae are needed.

Buzzing behavior may be carried over to other flowers during the same visitation bouts when bees shift from flowers with poricidal anthers to non-poricidal flowers that provide nectar (Buchmann 1985). In addition, Buchmann (1985) suggests bees may increase their collection of pollen by buzzing flowers with non-poricidal anthers. Usually on flowers with clustered stamens of long filaments and versatile anthers such as *Rosa*.

Other bee behaviors that release pollen. A variety of other behaviors may be exhibited by bees to release pollen from anthers, including those with poricidal anthers. Many bees are able to remove pollen from poricidal anthers without buzzing them. These are generally small bees and their behaviors have variously been described as "digging," "probing," "biting," "milking," "stroking," "drumming," or "striking." Some of these behaviors may result in pollen robbing. Anderson and Symon (1988) found that small stingless bees, *Trigona* (Apidae), unlike larger bees that sonicate *Solanum*, collected pollen by "digging it out of the terminal pores and by scavenging for it on floral parts such as the corolla and stigma." *Trigona* have been observed to alternately probe pollen from the apical pores of Melastomaceae with their tongues and then bite off the end of the anther to reach more pollen (Renner 1983, 1989).

In cranberry flowers, females of *Megachile addenda* grasp the staminal column with their forelegs and sometimes with their mandibles, so that the tips of the poricidal anthers press against their mid-venter. The bees then "vigorously stroke" the anthers with their mid and hind legs to release pollen (Cane et al. 1996). Honey bees drum the anther tips of the flowers with their forelegs to release pollen from the apical pores (Cane et al. 1993). Torchio (1990) describes a similar behavior on blueberry, which has similar flowers, by *Osmia ribifloris*.

The bee inserts her forelegs into the floral "bell" and makes rapid movements of them, thereby "striking" the anthers while rotating about half way round the flower, and releasing pollen.

Behaviors that appear to enhance pollen removal from flowers with non-poricidal anthers have been variously described as "scrabbling," "dancing," "mandibular scraping," and "patting" or "tapping" the metasomal venter on the anthers. Scrabbling is one of the more general terms that refers to the common movements of bees over a plane of anthers, such as in brush flowers or on the capitula of Apiaceae or Asteraceae. "Pollen dances" have been described for several species of oligoleges visiting species of *Clarkia* by MacSwain et al. (1973). Anthers of *Clarkia* are unique for Onagraceae in that they do not dehisce all at once, but present pollen gradually over one or more days. The dance motions of the bees presumably enhance release of pollen through mechanical stimulation of the anthers. Neff and Rozen (1995) describe the mandibular scraping of pollen from anthers of *Passiflora* (Passifloraceae) by the panurgine andrenid *Anthemurgus*. The female crawls up a stamen hangs beneath the anther grasping it with her mid and hind legs, then scrapes pollen from the stamen with her open mandibles. Pollen that accumulates on her mandibles, labrum and clypeus is groomed and packed. Acquisition of pollen by patting or tapping the metasomal venter on anthers has been described for *Macropis* (Cane et al. 1983), *Dieunomia* (Minckley et al. 1994) and some *Osmia* (Cripps and Rust 1989).

Combinations of structural and behavioral modifications to release pollen. Females of *Leioproctus macmillani* (Colletidae) in Western Australia use a distinct approach to extract pollen from non-poricidal anthers of *Astrolobma xerophyllum*. They thrust their foretarsi into the narrow corolla tube and use a combination of buzzing and alternately pumping the forelegs to draw pollen out of the tube where the midlegs pack the extracted pollen into the scopae (Houston 1991a). In his description of the female, Houston refers to

the "sparse, long, curved bristles of the dorsal and outer surface" of the foretarsi as contrasting with the "dense, short, fine setae of the inner surface." This is reminiscent of the structures in andrenid bees such as *Calliopsis* (*Verbenapis*), that are used to extract pollen from the narrow tubular flowers of *Verbena* (Shinn 1967).

Müller (1995) verified the use of hooked hairs on the forelegs of *Colletes nasutus* and on the mouthparts of *Andrena nasuta*, *Osmia pilicornis* and *Anthophora acervorum* for pollen collection by observing the probing and extraction behavior of bees at flowers of Boraginaceae. Although the *Osmia* and *Anthophora* are not oligoleges, they exhibit strong preferences for pollen from Boraginaceae. The *Anthophora* also used the same hairs and behavior to collect pollen from Primulaceae.

Specialized hairs on the heads of some bees are used to enhance pollen release from nototribic flowers. Megachilid bees with modified facial hairs have been observed to rub the hairs of the face over the anthers with "rapid back and forth movements" (Müller 1996a). Females of *Anthophora furcata* sonicated the anthers while holding their head against them, while females of *Rophites algirus* combined the two behaviors (Müller 1996a).

Females of *Panurginus atriceps* actively brush their heads against the trigger hairs of the nototribic anther column of *Downingia cuspidata* (Campanulaceae, Lobelioideae) in an up and down "bobbing" motion which results in pollen being liberally dusted on the face and dorsum of the mesosoma (Thorp 1990). Although I observed and documented this behavioral pattern in females of this bee many times, I did not recognize that there was an associated structural modification of the clypeal hairs until I reexamined female specimens in preparation for this review. Females (in the R. M. Bohart Museum of Entomology at the University of California, Davis) that were collected on *Downingia* have simple, stiff, downward directed clypeal setae that are different from the surrounding branched hairs and from the clypeal hairs of other *Panurginus*

species examined. Many of the females have residual *Downingia* pollen on the clypeus below the specially modified hairs indicating that these hairs aid in pollen removal.

Following this discovery, I examined the facial hairs of females of *Habropoda laboriosa* and compared them with those of five other *Habropoda* species. This was stimulated by research of Cane and Payne (1988) in which they showed that areas of the lower face, especially the labrum, and bases of the mouthparts of *H. laboriosa* are the sites that initially bear most of the pollen removed from blueberry flowers. I found that the hairs of the labrum on *H. laboriosa* appear shorter stouter and have shorter branches than those of other *Habropoda* species; the same seems true of the setae on the clypeus, but these are quite worn in most of our specimens. This feature should be more closely examined to determine whether this is another example of facial hairs modified for pollen removal.

Considerations for commercial pollination

Honey bees are our principal commercial pollinators, but they may not be the most efficient pollinators of some crops (Westerkamp 1991). This may be due to frequent grooming and packing pollen out of reach of stigmas by pollen foragers or pollen thieving from flowers structurally adapted to other pollinators. During conditions of pollen stress on sunflower, they may become secondary pollen thieves robbing pollen from scopa of other bees (Thorp and Briggs 1980). The examples cited by Westerkamp (1991) primarily concern naive nectar foragers that quickly learn to harvest nectar more efficiently by avoiding the sexual parts of the flower (apples) or special trip mechanisms that expose them (alfalfa). On the other hand, pollen foraging honey bees may be quite efficient in pollination the same crops (Free 1993). My own studies in almond suggest the same thing (Thorp 1979a and unpublished). However, the percent of pollen foragers returning to the hive from these crops is often quite low. Thus, manipulations

of the colony to increase pollen foraging, especially the use of bee pheromones (Pankiw et al. 1998) and genetic selection for increased pollen hoarding (Page and Fondrk 1995) may prove useful in improving pollination efficiency of colonies rented for commercial pollination in many crops where fruit and seed production has been less than desired (Gordon et al. 1995).

Some commercial crops remain difficult to pollinate using honey bees alone. Emphasis is being placed on the search for other bee species suitable as alternative or supplemental commercial pollinators for specific crops. *Megachile rotundata* and *Osmia lignaria* have been successfully used as a commercial pollinators of alfalfa seed and orchard crops respectively in western North America (Torchio 1991). Since honey bees do not sonicate flowers, considerable interest has focused on commercial pollination of buzz pollinated crops, especially greenhouse tomatoes. During the past 10 years interest has focused on *Bombus terrestris* and other bumble bees, due to the technological breakthrough of being able to produce large numbers of bumble bee colonies year round (Ruijter 1997). Other crops with poricidal anthers occur in the Ericaceae (blueberries and cranberries) and Actinidiaceae (kiwifruit), where bee visitors that sonicate the flowers to release pollen are of prime interest (Cane et al. 1985, Cane and Payne 1988, Corbet et al. 1988, Batra 1994, MacKenzie 1994). However, bees that use other methods to release pollen from these flowers may also be effective pollinators (Torchio 1990; Cane et al. 1993, 1996; Goodwin and Steven 1993) and should be considered as well.

Summary

Most pollen removal by flower visitors, including bees, is passive, especially in the case of bees seeking nectar or flower rewards other than pollen. Bees present a special case of flower visitors in that they are central-place foragers that use pollen as the primary food

for their offspring. As a result, the majority of bees have evolved specialized structures for transporting pollen to their nests and have modified their grooming behaviors to transfer pollen from their bodies to the transport structures. Bees that collect pollen passively and then discard it do so by using the final step in the ancestral grooming behavior for ridding the body of debris, rather than the derived apoid behavior of loading the pollen transport apparatus.

A variety of behaviors may be used to acquire pollen actively from flowers, depending on the repertoire of the bee and the structure of the flower, especially its anthers or the presence of any secondary pollen presentation mechanisms. Some bees display modified behaviors to release pollen from flowers that show adaptations for controlled emissions of pollen, such as those with poricidal anthers. The associated buzz pollination behavior is used not only on the many plants with poricidal anthers, but on other flowers as well. A few bees have developed both specialized structures (especially hairs) and associated behaviors for removal of pollen from plants that either hide their pollen in deep tubes (e.g. Boraginaceae), or deposit pollen on "safe sites" of visitor bodies (e.g. nototribic flowers of the Lamiaceae, Scrophulariaceae, Lobelioideae). The evolution of these specialized pollen removal structures has occurred several times independently in widely separated taxa, furthermore it is not restricted to oligoleptic bees, but also occurs in several polyleptic species.

On the practical side, as we search for better pollinators for our agricultural crops, we need to understand the various adaptations for pollen collection that may be present in the candidate species and how these adaptations relate to the broader pictures of both the pollen transfer within plant populations and the role of pollen in the bee's reproduction.

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Pollen morphological evolution in bat pollinated plants

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Abstract. This study assesses to what extent bat pollination has acted as a selective force on pollen morphology. Earlier pollen studies have suggested convergent evolution of verrucate exine ornamentation. Furthermore pollen of bat pollinated plants has been reported to be bigger than that of plants pollinated by other means. The generality of these ideas is tested using a sample of 130 species of both bat pollinated plants and relatives with another mode of pollination. An analysis of pollen size, shape, aperture number and type, and ornamentation type of 35 plant groups in which a transition towards bat pollination occurred was performed and showed a significant effect for pollen size only. Bat pollinated plants have bigger pollen than their relatives. Pollen size was shown to correlate with style length. Pollen shape, aperture system and exine ornamentation are in general not very different in bat pollinated plants in comparison with their relatives. There is no consistent trend for rougher exines to be associated with bat pollination.

Key words: Pollen morphology, chiropterophily, exine ornamentation, style length, pollination syndromes.

The study of pollen morphological evolution

The exines of angiosperm pollen grains exhibit a remarkable diversity in their surface sculpturing. This diversity has been used widely in systematics. Therefore most studies in pollen

morphology have tended to be descriptive in nature and do not always address evolutionary questions, although trends from primitive to advanced are sometimes discerned. It has, however, often been noted that exine patterns encountered in unrelated taxa can be strikingly similar. Especially if the resembling structures are of a well circumscribed, narrowly defined type this calls for an explanation. Such explanations can roughly be sought in three ways. Sometimes the seemingly low number of possibilities in which formation of patterns in similar condensation processes on spheroidal objects can take place is stressed, referring to patterns found for instance in precipitated glycerin jelly or produced with polystyrene (van Uffelen 1991, Hemsley et al. 1996). Modelling the process of exine formation then is a way to uncover factors that might influence this patterning process and the variation that results from it. A second option would be a microevolutionary approach: to try to quantify the effect on reproductive success of the structures under study. This would involve experimentally modifying pollen ornamentation and has not been done so far. The third approach is trying to assess the significance of the structures under study in an evolutionary context by correlation with the macromorphological differences associated with different pollination mechanisms. This should actually

be done within a phylogenetic framework. Significant correlation of independent character state changes on branches in a cladogram can indicate a causal relationship between features (Felsenstein 1985, Harvey and Pagel 1991). Linder (1998) did such an exercise in order to describe pollen morphological evolution associated with changes to wind pollination on the family level. So far however for studies relating to many different genera scattered over many families this method is hampered by the paucity of detailed and reliable cladograms. Therefore hardly any study on the evolutionary significance of pollen characteristics bases its claims on similarities in character evolution, depicted as state changes on cladograms.

Pollinator influenced pollen morphology and chiropterophilous plants

Transport by different kinds of pollinators is considered one of the factors that might have contributed to pollen diversification. A number of studies exist that describe optimal pollen size, shape and ornamentation for abiotic means of pollination (Crane 1986, Cox et al. 1991), or that compare biotically transported pollen with pollen from anemophilous plants (e.g. Osborn et al. 1991). Wodehouse (1935) already observed a loss of sculpturing in Compositae that reverted to wind pollination. In the domain of zoophily, evidence for pollinator influenced pollen morphologies is given by Grayum (1986). He finds echinate grains within the Araceae to be correlated with fly pollination, whereas beetle pollinated Araeaceae have psilate grains. Also pollination by larger beetles apparently selected for larger pollen size and permanent tetrad formation in this group. The role of the spinulae is further elaborated by Chaloner (1986) in terms of electrostatic properties that enhance pollination success.

For bat pollinated plants several authors found correlated pollen characteristics. Chavez (1974) studied 34 Mexican plant species in 29 genera spread over 19 different families.

His results indicate that bat pollen is very big and that the exine ornamentation is elaborate, resulting in an overall rough surface. The sample contained a wide variety of ornamentation types including striate, foveolate, reticulate, verrucate, gemmate and echinate grains.

Taylor and Levin (1975) found a significant relationship in the Polemoniaceae between large pollen and transportation by bats, but observed no apparent association with exine architecture or sculpturing. Graham and Barker (1981) have shown that in Caesalpinoideae a strikingly similar verrucate ornamentation type is present in genera with bat pollinated representatives like *Dicymbe*, *Elizabetha* and *Eperua*. Ferguson (1984, 1985) and Ferguson and Skvarla (1982) reported a correlation in the Papilioideae between verrucate ornamentation and bird or bat pollination. Ferguson (1990) furthermore signalled similar effects in neotropical representatives of *Mucuna*. Klitgaard and Ferguson (1992) compared bird, bat and moth pollinated *Brownea* and *Browneopsis* species, on the basis of which they concluded that the verrucate ornamentation in *Browneopsis* is best viewed as an adaptation to bat pollination. Although bird pollinated *Brownea* species failed to show a similar change, supposedly due to a constraint, Klitgaard and Ferguson see the overall pattern in this group as further proof for the association between verrucate exines and bird or bat pollination. Also in *Bauhinia* exine ornamentation was shown to be coupled at least partly with bat pollination (Ferguson and Pearce, 1986). Thus there seems to be evidence for bat adapted pollen size and ornamentation. However Dobat (1985), in his accurate overview of bat pollination, refuted this notion, stating that the patterns observed cannot be generalized. The purpose of the present study is to find out to what extent bat pollination adapted species in general display similarities in pollen characteristics. That would indicate convergent evolution and show that this pollination syndrome has acted as a selective force on pollen morphology.

The study group

Bat pollination is reported for roughly 750 species, dispersed over 270 genera and 64 families (Dobat 1985). Not all of these show clear adaptations to chiropterophily, some being without doubt only occasionally visited by bats but often pollinated otherwise. The classic work of Faegri and van der Pijl (1979) summarises the adaptations of bat pollinated plants in eight points: nocturnal anthesis, drab or whitish flower colours, pungent odour, strong flowers or inflorescences, large quantities of nectar, nectar position accessible to bats, large quantities of pollen, and an exposed position of the inflorescence. Dobat amply documents the variation found in bat flowers and in the characteristics mentioned, but subscribes to the notion that in general the flowers can be recognised on the basis of their morphology and display. The list of (possibly) bat pollinated plants given by Dobat (1985) is based on inference from flower morphology, observation of visits or the occurrence of pollen on fur or in bat dung. In the present study only those plants are considered that display most of the flower morphological adaptations mentioned and are reported to be visited by bats.

Methods

Pollen data of 130 plant species from 50 genera and 23 families was gathered from literature or from direct observation. In the latter case pollen was taken from herbarium specimens and acetolysed (except for the ones not resistant to acetolysis). Of the studied species, 75 are bat pollinated, the remaining 55 are relatives that are pollinated by other means. Together the sample comprises 35 plant groups in which species with bat pollination as well as other pollination mechanisms are present. A comparison within these 35 groups was made between the bat pollinated species and the others for unit of dispersal, pollen size, shape, aperture system and ornamentation type. The largest diameter was taken as a measure for pollen size and thus can represent either a polar or equatorial value. The terminology used follows Punt et al. (1994). Pairwise differences in pollen size between the two groups were tested using a t-test

(paired two samples for means), for which the means of the bat pollinated species and the means of the others per group were calculated. Grain size for tetrads or polyads was not measured and unit sizes given for the species concerned were left out of the analysis.

Style length is given for 72 species and either taken from literature, or measured in at least 10 flowers from herbarium specimens. The difference in style length between bat and non-bat plants was tested using a t-test for samples with unequal variances. A regression analysis between pollen size and style length was performed to find out to what extent the two are correlated.

For those 19 (out of the 35) groups for which sufficient style length measurements were present, a pairwise comparison of bat and non-bat species was made between pollen size increase/decrease and style length increase/decrease. The null hypothesis that increase in pollen size is coupled with increase in style length (or decrease with decrease) was tested with a sign test.

Pollen size differences between bat and non-bat flowers were also tested after correction for style length variation by testing the ratios between the two. This was done in order to eliminate any style length effect and to see if apart from it differences between bat plants and others could still be found.

Ornamentation types were classified following Punt et al. (1994), sometimes with specifications like finely or coarsely. The number of occurrences of the verrucate, coarsely verrucate and coarsely reticulate ornamentation types together was compared between bat pollinated plants and non-bat plants and tested with a G-test (=log-likelihood ratio test, see Sokal and Rolf 1995). Since no measure of overall exine roughness exists or could be developed easily, ornamentation was compared on the basis of a qualitative judgement in which the bat pollinated plants in a genus were scored as distinctly rougher than, more or less similar to, or smoother than their relatives. Pollen shape and aperture system are evaluated qualitatively.

Results

Sizes. Characteristics of the species are given in appendix 1. It was found that pollen of bat plants is generally big, on average 72 µm ($n = 75$), whereas the pollen of the relatives was significantly smaller: 64 µm ($n = 55$,

$P(T > t \text{ one-sided}) = 0.010$). This latter size is still quite big in comparison with most angiosperms. Pollen in bat pollinated plants varied between the tiny pollen of *Cleome anomala* (17 μm) and the quite large grain of *Cobaea aschersoniana* (170 μm). The range found in relatives is slightly bigger: 17–193 μm . Also style length in bat plants was found to vary greatly, (4–240 mm). Bat plants have long styles, on average 52 mm ($n = 44$), though this is influenced by a few outliers in the Malvaceae (*Adansonia*, *Pachira*, *Pseudobombax*). The mean style length of the relatives studied is 42 mm. ($n = 28$) which is significantly shorter ($P(T > t \text{ one-sided}) = 0.04$).

In Fig. 1 the pollen size of all plants investigated is plotted against the style length. The regression analysis showed that the data fit a multiplicative model best. In this model pollen size is calculated from style length by the following formula: pollen size = $16.7 * (\text{style length})^{0.32}$. The correlation pollen size and style length is moderately strong and significant (correlation coefficient = 0.56, $P < 0.01$) but as can be seen the variation in pollen sizes is big and only for a part explained by differences in style length ($r^2 = 0.31$). In

Fig. 1 it can furthermore be seen that plants with pollen over 100 μm do not seem to fit in nicely. If these are left out of the analysis the results are the same. The outliers with the big pollen are *Cobaea* (Polemoniaceae) and *Louteridium* (Acanthaceae) species.

A sign test on increase/decrease patterns for pollen size change in relation to style length change for 19 within group comparisons showed that in 14 cases in- or decrease of pollen size and style length were coupled and that 2 positive and 3 negative differences were found. This is clearly supporting the null hypothesis that in- or decreases are positively coupled between pollen size and style length.

When pollen sizes are corrected for differences in style length by dividing pollen size through style length, the difference between bat and non-bat plants is no longer significant ($P(T > t \text{ one-sided}) = 0.17$) for this data set.

Ornamentation. Ornamentation in bat pollinated species virtually spans the whole range of main ornamentation types (examples: Fig. 2 A–F). The type of ornamentation was found to be quite constant within the 35 groups regardless of pollination mode. Within-group differ-

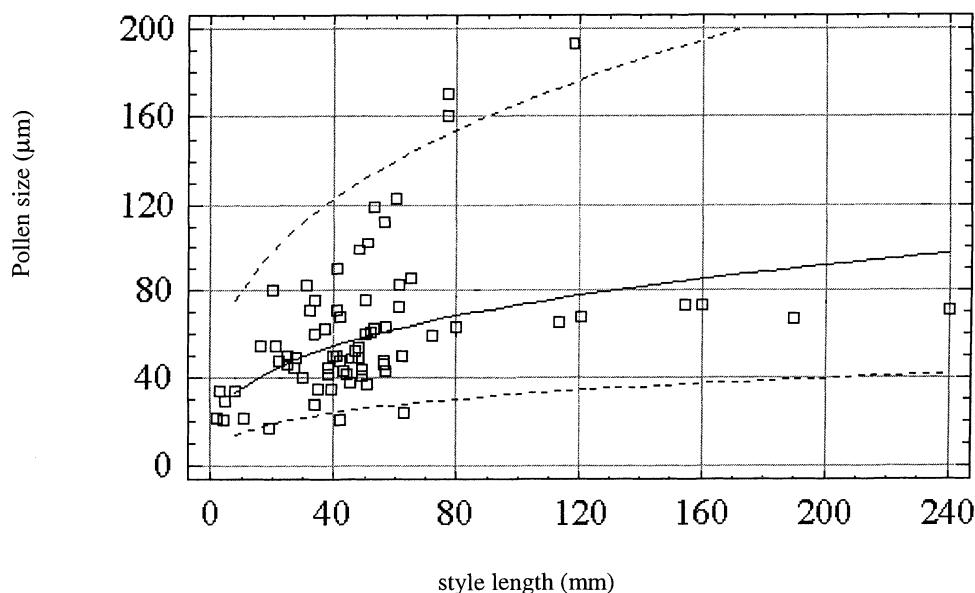


Fig. 1. Correlation between pollen size and style length. Dotted lines are prediction limits

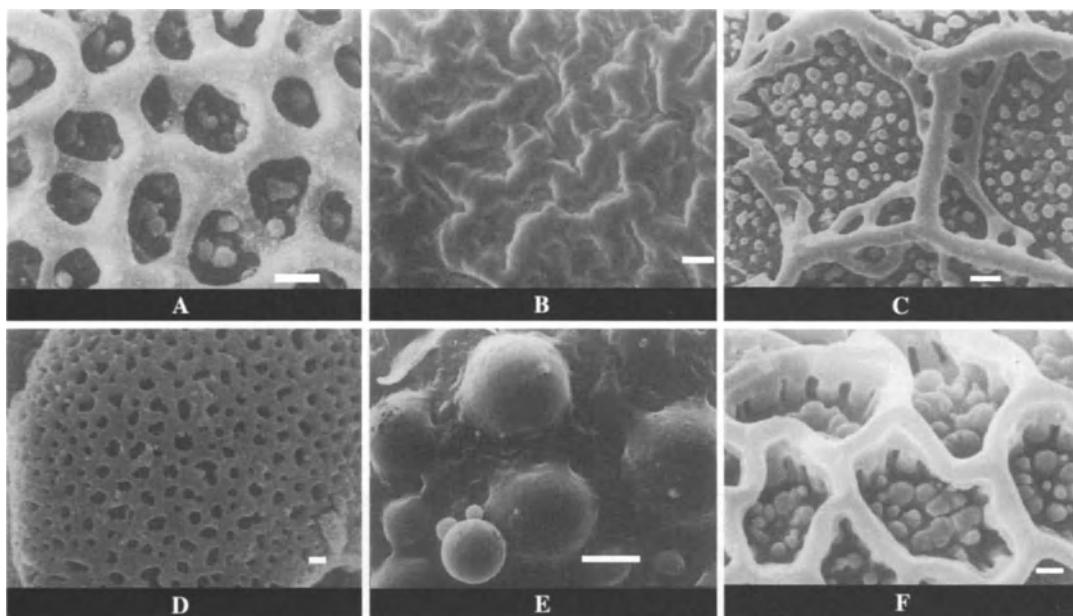


Fig. 2 A–F. Different ornamentation types encountered in bat pollinated plants. **A** *Bombax ceiba*; **B** *Caryocar pallidum*; **C** *Ceiba pentandra*; **D** *Lecythis poiteauii*; **E** *Durio oblongus*; **F** *Passiflora mucronata* (scale bars 1 µm, in E 5 µm)

ences were only detected in 13 of the 35 comparisons, and those are mainly between species that represent speciose genera (e.g. *Bauhinia*, *Cleome*, *Mucuna*). In most groups no clear difference in ornamentation type was found to exist between bat pollinated plants and their relatives though important exceptions may be found in the Caesalpinioidae, Capparidaceae and Caryocaraceae. A comparison of the ‘rough’ ornamentation types putatively associated with bat pollination (verrucate, coarsely verrucate and coarsely reticulate) versus the other ornamentation types in bat and non-bat plants showed that 23 out of 77 bat pollinated species had one of these rough types, against 12 out of 54 for the non-bat plants. A G-test indicates that this is not a significant difference. This result is reflected in the analysis of roughness differences as well. Only in 8 out of the 35 groups the bat plants had a rougher exine sculpturing than the others, while in 5 comparisons the opposite was found. This also means that 22 times no distinct difference was observed.

Apertures and shape. The well known conservative nature of aperture system on genus level was confirmed in that only minor variation was found in this characteristic, none of which can be meaningfully discussed in relation to pollination modes. Pollen shape is less conservative (17 within-genus differences) but it does not show any relation to the distinction of bat versus not-bat pollinated. It is also important to note that within-species variation can be considerable, but this aspect was left out of the comparison. Changes from monad to tetrad or polyad (or vice versa) were not found. Tetrad or polyads occur mainly in the legumes (*Calliandra*, *Inga*), in *Bauhinia* two species with tetrads are known.

No cases of polymorphic pollen were observed in bat pollinated plants. Some species in genera included in this study, like *Lagerstroemia*, have dimorphic pollen but these are not the bat pollinated ones. Within the Lecythidaceae *Couroupita guianensis* pollen is dimorphic with tetrads that are found in the anthers of the androecial hood, whereas monads are found in the anthers of the staminal

ring (Mori et al. 1980). Pollen polymorphy generally seems to be restricted to entomophilous plants, maybe even exclusively so to the bee pollinated ones.

Data on the ornamentation in selected groups

Positive evidence for a relationship between bats as pollinators and pollen ornamentation is found in the Caesalpinoideae, Caryocaraceae and maybe in *Cleome* (Capparidaceae, Fig. 3A–B) and *Irlbachia* (Gentianaceae)

(Fig. 4N). The latter two groups however suffer from a lack of data on pollination modes, and in *Irlbachia* the unclear affinities with species of the putatively related genera (*Chelonanthus*, *Lisianthes*) hamper a good comparison. In *Caryocar* almost all species are supposed to be bat pollinated. Evidence is given by Vogel (cited from Dobat 1985) for *Caryocar brasiliense*, *C. glaber* and *C. villosum*. The only probable exception (Prance and Freitas da Silva 1973), *Caryocar gracile*, does differ with respect to exine roughness. Its

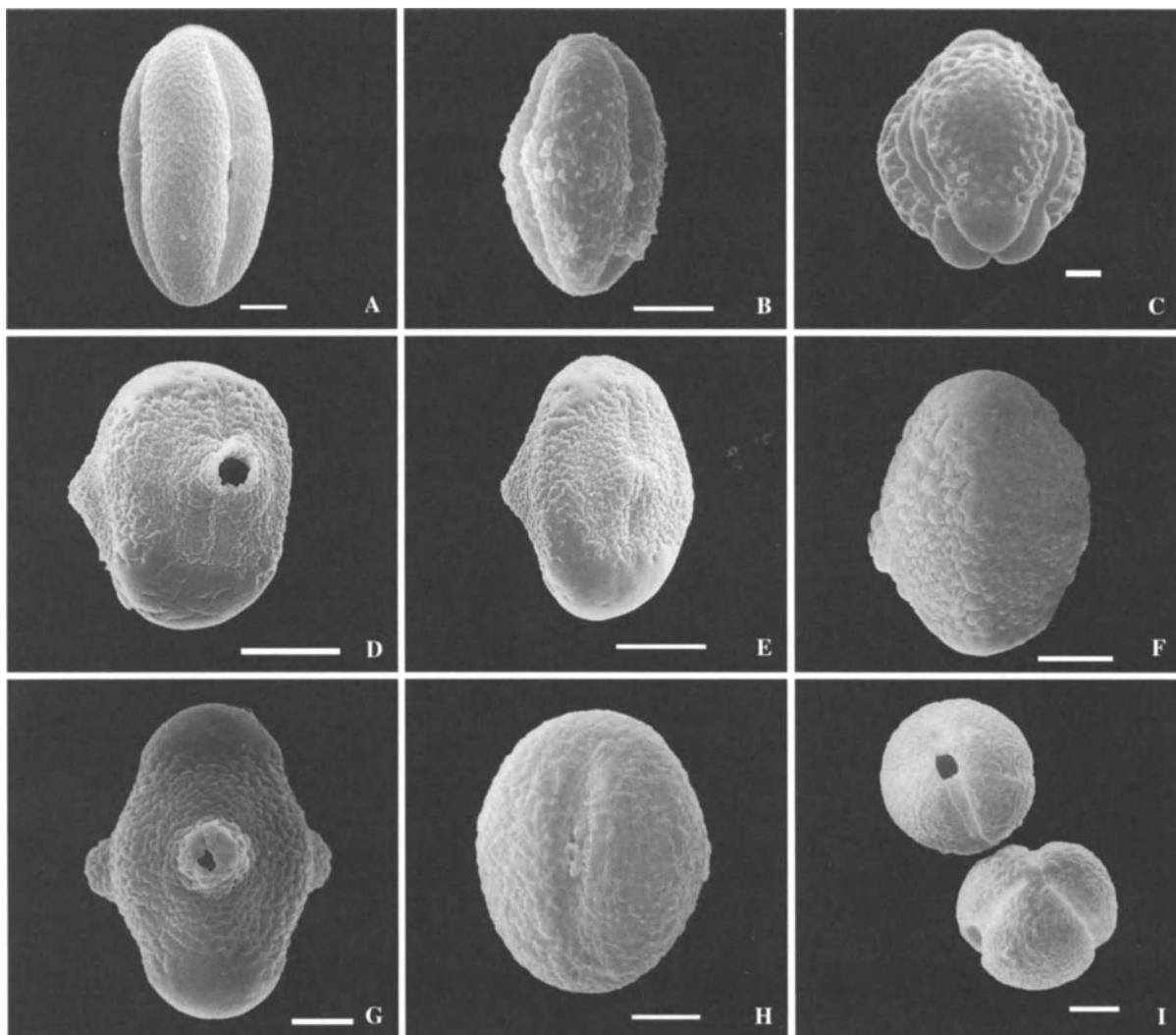


Fig. 3 A–I. Pollen grains of bat pollinated plants and their relatives. **A** *Cleome guianensis*; **B** *Cleome moritziana*; **C** *Chydenanthus excelsus*; **D** *Lafoensia pacari*; **E** *Lafoensia replicata*; **F** *Duabanga grandiflora*; **G** *Sonneratia caseolaris*; **H** *Punica granatum*; **I** *Lecythis poiteau* (all scale bars 10 µm)

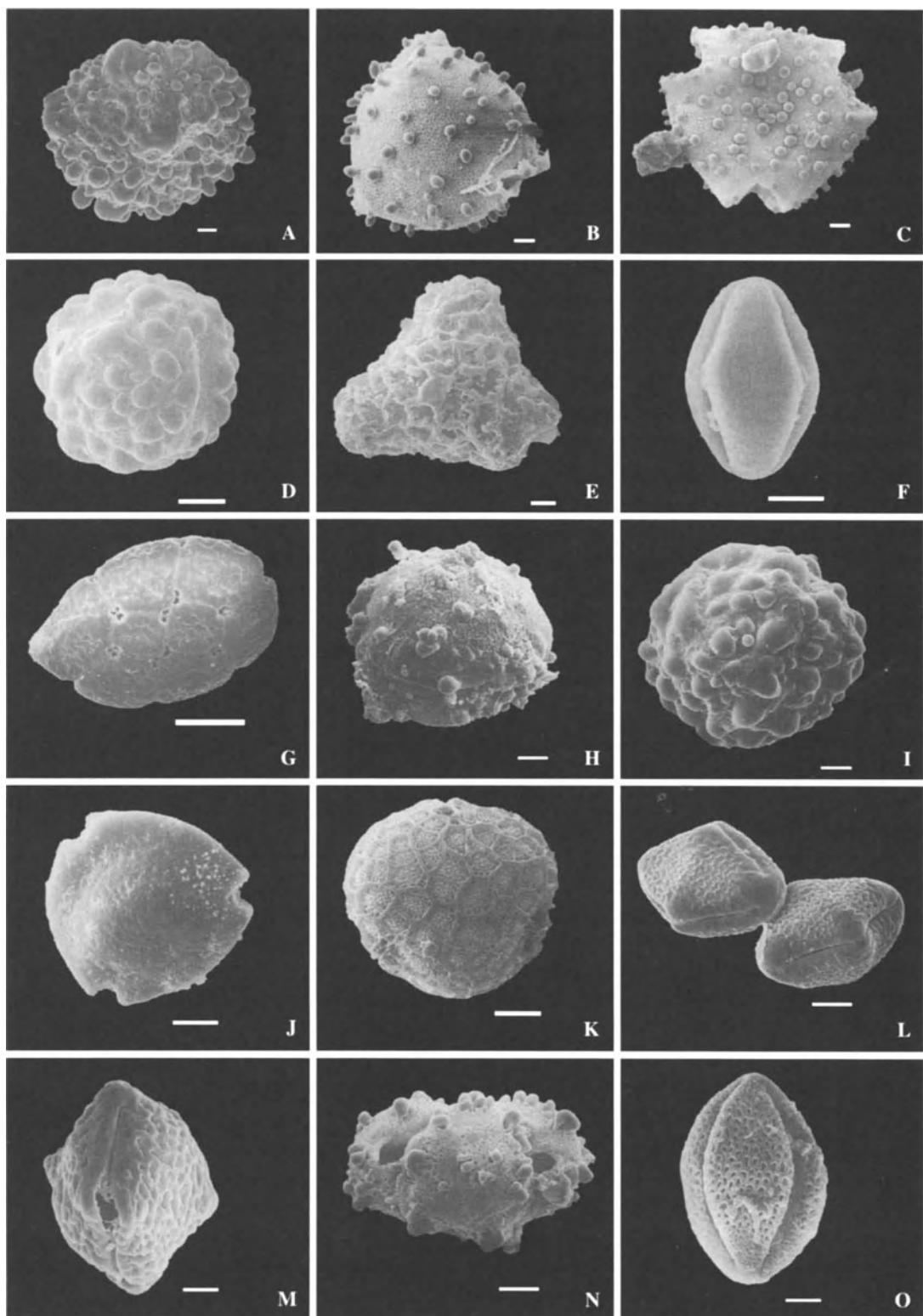


Fig. 4A–O. Pollen grains of bat pollinated plants and their relatives. **A** *Bauhinia macrostachya*; **B** *Bauhinia megalaundra*; **C** *Bauhinia rufa*; **D** *Elizabetha paraensis*; **E** *Eperua falcata*; **F** *Hymenaea courbaril*; **G** *Calliandra confusa* (polyad) **H** *Durio kutejensis*; **I** *Durio oblongus*; **J** *Durio zibethinus*; **K** *Ceiba pentandra*; **L** *Caryocarp pallidum*; **M** *Caryocar villosum*; **N** *Irlbachia alata* (tetrad); **O** *Oroxylum indicum* (all scale bars 10 µm)

mesocolpal ornamentation is psilate, instead of the coarsely areolate to reticulate patterning commonly found in the other species of *Caryocar* (Fig. 2B, 4L–M, Barth 1966). *Anthodiscus*, the only other genus in the Caryocaraceae, is entomophilous and has small and smooth pollen grains that differ from those of *Caryocar* in many respects.

Graham and Barker's hypothesis that bat pollination influenced pollen morphology in the Caesalpinoideae seems to be supported by the genera *Elisabetha* (Fig. 4D) and *Eperua* (Fig. 4E), but not by *Hymenaea* (Fig. 4F). Correlation within *Bauhinia* (Figs. 4A–C) is fair (though only neotropical) and is supported by the species selected for this study. In the other legume families no support could be found (*Calliandra*, 4G).

Negative evidence is found in other important families. Malvaceae seem to display no correlation of pollination type and pollen characteristics whatsoever. For instance in *Adansonia* there are 8 species of which 2 are bat pollinated and 1 is lemur (and bat) pollinated (Baum et al. 1998). The variation found in tectum and spines in this genus is not correlated with pollination mode. The characteristic coarse reticulum (Figs. 2C, 4K) in *Ceiba pentandra* is also found in a.o. *Spirostheca*, which is not pollinated by bats (Nilsson and Robyns 1986). In *Bombax* the reticulum found in *B. ceiba* (Fig. 2A) is found in other species as well, sometimes with wider lumina like in *B. costatum*. Variation in exine.sculpturing is quite substantial in *Durio* (Fig. 4H–J). The well known bat pollinated *Durio zibethinus* and *Durio graveolens* are at the smooth end of the spectrum, while other bat pollinated durians are verrucate (*D. kutejensis*). In many families no bat pollination-correlated differences can be shown. The Bignoniaceae, a very important family for nectarivorous bats, can serve as an example for that. Three bat pollinated Bignoniaceae genera, *Kigelia*, *Oroxylum* and *Pajanelia*, are monotypic and their phylogenetic affinities are not clear. Pollen morphologically they are not very conspicuous within this family. *Oroxylum indicum* (Fig. 4O) has very

large grains, with no apparent rough exine sculpturing however, which is in contrast with Buurman (1977) who described it as being roughly verrucate. Perhaps the most ornamented (and also extremely large) pollen within the Bignoniaceae is found in *Nyctocalos*. The remarkable pollen morphology of this genus is described by Ferguson and Santisuk (1973). Species of *Nyctocalos* are reported to be bat pollinated (Dobat 1985) but its flower morphology and position suggests an adaptation to nocturnal Lepidoptera. *Dolichandrone* contains other examples of plants mistakenly believed to be bat pollinated. In fact the only ones that are, belong to the genus *Markhamia*. The pollen of these two closely related genera is similar. In the Lecythidaceae the pollen of *Barringtonia* is very characteristic, being syncolpate with well developed marginal ridges along the colpi. It is however, very similar to that of other genera in the Planchonioidea, in particular grains of the genera *Abdulmajidia*, *Chydenanthus* (Fig. 3C) and *Petersianthus* (Tsou 1994) and *Foetida*. None of these shows any bat pollination related flower characteristics.

For the completely bat pollinated genus *Sonneratia*, a comparison had to be made with its closest non-bat pollinated relatives in the lythraceous genera *Punica* (*Punica granatum*, Fig. 3H), *Lagerstroemia* and *Lawsonia*. It does not show a marked difference in exine roughness. These groups were studied extensively by Muller (1981) and Graham et al. (1990). The peculiar pollen morphology (see 3F–G) is explained by Muller in terms of harmomegathy. Interestingly, it is also found in another more distantly related bat pollinated Lythracean genus, *Lafoensia* (Fig. 3D–E).

A special case is represented by the monocots that are bat pollinated. Many of them have grains possessing a very reduced exine and a much elaborated intine. This is known to occur in many unrelated families within the monocots. It does not appear to be associated with pollination syndromes, but is better 'explained' in terms of phylogeny (Kress 1986). Most of these species seem to have psilate grains. Interestingly, there seems to be a

group of species, like *Ensete ventricosum* (Musaceae), of which the pollen is ornamented with verrucae which are separate ectexinous elements. This deserves further study since it could be speculated that if the rest of the exine was lost but the verrucae were not, then they might have been maintained by selection.

Discussion

The results indicate that in general switches towards bat pollination seem to have led to bigger pollen, but did not influence exine morphology. The size effect is in accordance with the results of Chavez (1974), though a considerable difference in the mean size for bat pollen in this study and that of Chavez exists: 72 versus 94 µm. This is partly explained by Chavez's inclusion of *Mirabilis longiflora* (Nyctaginaceae) which has pollen grains measuring 272 µm on average. Based on the long and very slender corolla tube with a narrow opening in this species I think it is pollinated by long tongued Lepidoptera instead.

There are several reasons why application of the conclusion that exine sculpturing is not influenced by bat pollination to a more specific subset of taxa might not be valid. As a wide variety of exine types was observed, it is likely that the other factors that are thought to influence evolution of the outside of pollen grains have led to phylogenetic constraints in some groups, which possibly prohibited adaptations to pollinators. As Crane (1986) noted it should be self-evident that pollen grains are not optimally designed for a specific function, but merely structures that work with varying efficiency in a specific ecological and evolutionary context. Of course this also holds for the transport function. Other important factors may include the storage capacity of compounds that play a role in self-incompatibility systems, pollen-stigma interaction, harmomegathy and other forms of protection against negative environmental influences. An example might be the above mentioned harmomegathic structure in *Sonneratia*. Thus an overview over many groups might not show the effects that

can be found in subsets of the data. However, this analysis shows that styles in bat plants are longer, which is explicable in terms of function. If a similar selective force was present for rough exine, it should have become evident from this overview as well.

A possibility that cannot be excluded is the presence of elements that make the outside of a grain rougher, and therefore more susceptible for uptake, but that are not acetolysis-resistant. As Stone et al. (1979) puts it: "from a functional point of view, the exine which has undergone acetolysis treatment is a skeleton devoid of the vital signs. Questions about the functional, adaptive, or evolutionary significance of pollen should consider the total life history of a pollen grain and be aware of the limitations imposed by the study techniques". In the case of pollination this concerns for instance the presence and amount of pollenkitt, which is outside the scope of this study. It is noteworthy that no threadlike structures were observed, since these could in theory enhance adhesion to bat hairs. But threadlike structures are mentioned in literature for genera to which bat pollinated species belong. They were found in *Jacqueshuberia* (Caesalpinoideae) by Graham et al. (1980) but there only on some grains, and few (Ferguson and Skvarla 1982). The structures found in *Jacqueshuberia* cannot be considered homologous with viscin threads (Patel et al. 1985), found in for instance *Oenothera* and *Fuchsia* (Onagraceae), but they might have a similar function. The few threads found in bat plants could however be the tip of the iceberg, since there are also thread like structures that are not acetolysis-resistant (see Hesse et al., this volume).

Conservative features in pollen morphology reflecting phylogeny more than function might seem strange. Since pollen plays a vital role in the reproductive process, how can its outside not show any characteristics determined by selection? Maybe the patterns observed in the Caesalpinoideae, for instance by Graham and Barker, do not represent sensu stricto adaptations, but exaptations (traits contributing to fitness, but not evolved as a

Appendix 1. Pollen characteristics for 75 bat pollinated plants and their relatives. Bat pollinated species are marked with a b, non-bat pollinated ones with a n. Approximate species numbers are given with the genus names. Abbreviations for unit are: m monad, p polyad, t tetrad

	Bat poll. or not	Species	Size (μm)	Ornamentation	Shape	Apertural configuration	Unit	Style length (mm)
Acanthaceae								
<i>Louteridium</i> (6)	b	<i>chartaceum</i>	123	gemmate	spheroidal	pantoporate	m	60
	b	<i>donell-smithii</i>	99	gemmate	prolate	pantoporate	m	48
	n	<i>costaricense</i>	102	gemmate	spheroidal	pantoporate	m	51
<i>Trichanthera</i> (2)	b	<i>gigantea</i>	38	regularly striate punctate	prolate	2-colporate	m	45
Agavaceae								
<i>Agave</i> (100+)	b	<i>palmeri</i>	82	reticulate	prolate	monocolpate	m	31
	b	<i>schottii</i>	80	reticulate	prolate	monocolpate	m	20
	n	<i>attenuata</i>	68	reticulate	prolate	monocolpate	m	42
<i>Polianthes</i> (8)	b	<i>bulliana</i>	62	reticulate	prolate	2-colpate	m	
	n	<i>geminiflora</i>	56	coarsely reticulate	prolate	2-colpate	m	
	n	<i>mexicana</i>	53	coarsely reticulate	prolate	2-colpate	m	
Bignoniaceae								
<i>Crescentia</i> (6)	b	<i>cuyete</i>	43	finely reticulate	prolate	3-colpate	m	43
<i>Kigelia</i> (1)	b	<i>africana</i>	41	reticulate	spheroidal	pantocolpate	m	49
<i>Markhamia</i> (10)	b	<i>stipulata</i>	50	finely reticulate	spheroidal	3-colpate	m	40
	n	<i>platycalyx</i>	50	finely reticulate	spheroidal	3-colpate	m	41
<i>Oroxylum</i> (1)	b	<i>indicum</i>	60	reticulate	prolate	3-colpate	m	50
<i>Pajanelia</i> (1)	b	<i>multijuga</i>	52	reticulate	prolate	3-colpate	m	47

Bromeliaceae								
<i>Puya</i> (168)	b	<i>ferruginea</i>	82	coarsely reticulate	heteropolar	monocolporate	m	61
	n	<i>chilensis</i>	60	reticulate	heteropolar	monocolporate	m	34
<i>Vriesea</i> (280)	b	<i>bituminosa</i>	71	reticulate	heteropolar	monocolporate	m	41
	b	<i>gladioliflora</i>	62	reticulate	heteropolar	monocolporate	m	37
	n	<i>friburgensis</i>	55	reticulate	heteropolar	monocolporate	m	16
	n	<i>jonghei</i>	50	reticulate	heteropolar	monocolporate	m	25
Caesalpinioidae								
<i>Bauhinia</i> (300+)	b	<i>macrostachya</i>	85	perforate verrucate	oblate	3-colporate	m	
	b	<i>megalandra</i>	112	verrucate	spheroidal	3-colporate	m	
	b	<i>paulista</i>	87	perforate	spheroidal	3-colporate	m	
	b	<i>rufa</i>	98	perforate	oblate	3-colporate	m	
	b	<i>siqueiraei</i>	94	verrucate	prolate	3-colporate	m	
	n	<i>guianensis</i>	53	psilate	prolate	3-colporate	m	
	n	<i>phoenicea</i>	107	micropelorate	spheroidal	3-colporate	t	
	n	<i>reflexa</i>	56	psilate	prolate spheroidal	3-colporate	m	
<i>Brownea</i> (12)	n	<i>grandiceps</i>	46	striate	prolate spheroidal	3-colporate	m	56
	n	<i>peruviana</i>	45	coarsely	oblate spheroidal	3-colporate	m	38
				verrucate				
<i>Browneopsis</i> (6)	b	<i>cauliflora</i>	49	coarsely	oblate spheroidal	3-4-porate	m	46
	b	<i>disepala</i>	50	verrucate	oblate spheroidal	3-colporate	m	62
	b	<i>macrofoliolata</i>	63	verrucate	oblate spheroidal	4-colporate	m	57
	b	<i>ucayalina</i>	48	coarsely	oblate spheroidal	4-porate	m	42
				verrucate				

Appendix 1 (continued)

	Bat poll. or not	Species	Size (µm)	Ornamentation	Shape	Apertural configuration	Unit	Style length (mm)
<i>Daniellia</i> (9)	b	<i>olivieri</i>	35	psilate	oblate spheroidal	3-synporate	m	
	n	<i>ogea</i>	46	psilate	oblate spheroidal	3-corporate	m	
<i>Elizabetha</i> (11)	b	<i>speciosa</i>	45	coarsely verrucate	spheroidal	3-porate	m	27
<i>Eperua</i> (15)	b	<i>falcata</i>	90	coarsely reticulate	oblite	3-(col)porate	m	41
	n	<i>leucantha</i>	71	rugulate	oblite	3-(col)porate	m	32
Capparaceae								
<i>Cleome</i> (150)	b	<i>anomala</i>	17	microechinate/ verrucate	prolate spheroidal	3-corporate	m	
	b	<i>arborea</i>	24	verrucate	prolate spheroidal	3-corporate	m	
<i>b</i>	<i>moritziana</i>		18	microechinate/ verrucate	prolate spheroidal	3-corporate	m	
	b	<i>viridiflora</i>	23	verrucate	spheroidal	3-corporate	m	
<i>b</i>	<i>aculeata</i>		24	finely microechinate	spheroidal	3-corporate	m	
	n			finely reticulate	prolate	3-corporate	m	
<i>n</i>	<i>guianensis</i>		29	finely reticulate	prolate spheroidal	3-corporate	m	
	n	<i>viscosa</i>	28	finely reticulate			m	
Caryocaraceae								
<i>Anthodiscus</i> (10)	n	<i>amazonicus</i>	34	perforate	prolate	3-corporate	m	3
<i>Caryocar</i> (15)	b	<i>villosum</i>	85	reticulate	prolate spheroidal	(3-)parasyncolpate	m	65
	b	<i>brasiliense</i>	75	areolate reticulate	prolate spheroidal	(3-)parasyncolpate	m	50
<i>n</i>	<i>gracile</i>		46	psilate	prolate spheroidal	(3-)parasyncolpate	m	25
Chrysobalanaceae								
<i>Couepia</i> (67)	b	<i>longipendula</i>	40	reticulate	spheroidal	3-corporate	m	
	n	<i>polyandra</i>	36	striate reticulate	spheroidal	3-corporate	m	

Cucurbitaceae								
<i>Calycophysum</i> (5)	b	<i>pedunculatum</i>	103	verrucate echinate	spheroidal	pantoporate	m	
<i>Cucurbita</i> (13)	n	<i>pepo</i>	160	echinate	spheroidal	pantoporate	m	
Gentianaceae								
<i>Iribachia</i> (17)	b	<i>alata</i>	57	coarsely verrucate		t		
	n	<i>pendula</i>	65	coarsely reticulate		t		
Lecythidaceae								
<i>Lecythis</i> (25)	b	<i>poiteui</i>	29	perforate reticulate	prolate	3-corporate	m	5
	n	<i>corrugata</i>	21	perforate	prolate spheroidal	3-corporate	m	4
	n	<i>tuyiana</i>	22	psilate perforate	prolate	3-corporate	m	2
<i>Barringtonia</i> (39)	b	<i>asiatica</i>	65	irregularly punctate	prolate spheroidal	3-syncolpate	m	113
	n	<i>calyptirata</i>	42	sparsely punctate	prolate spheroidal	3-syncolpate	m	38
	n	<i>calyptrocalyx</i>	40	punctate areolate	prolate spheroidal	3-syncolpate	m	30
	n	<i>papuana</i>	35	punctate areolate	prolate spheroidal	3-syncolpate	m	35
Lythraceae								
<i>Duabanga</i> (2)	b	<i>grandiflora</i>	24	verrucate	prolate spheroidal	3-porate	m	63
	b	<i>moluccana</i>	21	finely verrucate	prolate spheroidal	3-porate	m	42
<i>Lafoensis</i> (10)	b	<i>pacari</i>	37	finely verrucate	prolate	3-porate	m	51
	b	<i>panicifolia</i>	44	finely verrucate	prolate	3-porate	m	49
	n	<i>replicata</i>	35	finely verrucate	prolate	3-porate	m	39
<i>Lagerstroemia</i> (53)	n	<i>inermis</i>	34	finely verrucate	prolate	3-porate	m	8
<i>Punica</i> (2)	n	<i>granatum</i>	22	finely verrucate rugulate	prolate	3-corporate	m	11
	n	<i>protopunica</i>	23	finely verrucate rugulate	prolate	3-corporate	m	
<i>Sonneratia</i> (6)	b	<i>apetala</i>	49	finely verrucate	spheroidal	3-porate	m	48
	b	<i>ovata</i>	42	finely verrucate	prolate spheroidal	3-porate	m	44
	b	<i>caseolaris</i>	48	verrucate	prolate spheroidal	3-porate	m	56

Appendix 1 (continued)

	Bat poll. or not	Species	Size (μm)	Ornamentation	Shape	Apertural configuration	Unit	Style length (mm)
Malvaceae (s.l.)								
<i>Adansonia</i> (8)	b	<i>digitata</i>	61	microreticulate with small spinulae	oblate spheroidal	3-porate	m	52
	b	<i>grandiflora</i>	59	microreticulate with small spinulae	oblate spheroidal	3-porate	m	72
	n	<i>perrieri</i>	67	microreticulate with small spinulae	oblate spheroidal	3-porate	m	190
<i>Bombax</i> (20)	b	<i>ceiba</i>	43	reticulate	oblate	3-colporate	m	57
	n	<i>costatum</i>	49	coarsely reticulate	oblate	3-colporate	m	28
<i>Ceiba</i> (11)	b	<i>pentandra</i>	75	coarsely reticulate	oblate	3-colporate	m	34
	n	<i>pubiflora</i>	48	reticulate	oblate spheroidal	5-colporate	m	22
<i>Durio</i> (28)	b	<i>graveolens</i>	54	psilate	spheroidal	3-colporate	m	48
	b	<i>kunzejensis</i>	63	sparsely verrucate	oblate spheroidal	3-colporate	m	80
	b	<i>zibethinus</i>	62	psilate	oblate	3-colporate	m	53
	n	<i>affinis</i>	55	psilate	oblate spheroidal	3-colporate	m	21
	n	<i>oblongus</i>	72	micronrugulate coarsely verrucate	spheroidal	3-colporate	m	61
<i>Ochroma</i> (1)	b	<i>pyramidalis</i>	83	reticulate	oblate spheroidal	3-colporate	m	
<i>Pachira</i> (20)	b	<i>aquatica</i>	71	reticulate echinate	oblate	3-colporate	m	240
<i>Pseudobombax</i> (20)	b	<i>grandiflorum</i>	73	reticulate	oblate	3-colporate	m	155
	b	<i>longiflorum</i>	73	reticulate	oblate	3-colporate	m	160
	n	<i>marginatum</i>	68	reticulate	oblate	3-colporate	m	121

Melastomataceae								
<i>Tibouchina</i> (243)	b	<i>grossa</i>	28	psilate	prolate spheroidal	3-corporate	m	34
	n	<i>longifolia</i>	17	psilate	prolate spheroidal	3-corporate	m	19
Mimosoideae								
<i>Calliandra</i> (200)	b	<i>confusa</i>	186	psilate fossulate			p	
	n	<i>glyphoxylon</i>	136	smoothly verrucate			p	
<i>Inga</i> (350)	b	<i>spectabilis</i>	160	verrucate			p	
	b	<i>vera</i>	145	rugulate			p	
	n	<i>minutula</i>	148	verrucate			p	
	n	<i>umbellifera</i>	93	verrucate			p	
<i>Parkia</i> (30)	b	<i>decussata</i>	120	verrucate			p	
	b	<i>pendula</i>	133	psilate sparsely perforate			p	
	b	<i>platycephala</i>	134	psilate sparsely perforate			p	
	n	<i>ulei</i>	69	perforate			p	
	n	<i>velutina</i>	150	areolate verrucate			p	
Musaceae								
<i>Musa</i> (35)	b	<i>acuminata</i>	53	psilate	prolate spheroidal	inaperturate	m	
	n	<i>halabensis</i>	46	psilate	prolate spheroidal	inaperturate	m	
<i>Heliconia</i> (150)	b	<i>solomonensis</i>	75	psilate	oblate spheroidal	inaperturate	m	
	b	<i>indica</i>	73,5	psilate/minutely & sparsely verrucate	oblate to prolate spheroidal	inaperturate	m	
	b	<i>papuana</i>	63	minutely & sparsely verrucate	oblate spheroidal	inaperturate	m	
	b	<i>lanata</i>	80	minutely & sparsely verrucate	prolate spheroidal	inaperturate	m	
	n	<i>laufao</i>	69	minutely & sparsely verrucate	oblate spheroidal	inaperturate	m	

Appendix 1 (continued)

	Bat poll. or not	Species	Size (µm)	Ornamentation	Shape	Apertural configuration	Unit	Style length (mm)
	n	<i>paka</i>	70	minutely & sparsely verrucate	oblate spheroidal	inaperturate	m	
Papilionoideae								
<i>Mucuna</i> (100)	b	<i>mutisiana</i>	78	reticulate	prolate spheroidal	3-colporate	m	
	b	<i>pruriens</i>	51	coarsely reticulate	spheroidal	3-colporate	m	
	n	<i>albertisii</i>	37	perforate	oblate spheroidal	3-colporate	m	
	n	<i>huberi</i>	43	coarsely verrucate	oblate spheroidal	3-colporate	m	
Passifloraceae								
<i>Passiflora</i> (430)	b	<i>mucronata</i>	98	coarsely reticulate	oblate spheroidal	parasyncolpate	m	
	n	<i>ambigua</i>	94	coarsely reticulate	prolate spheroidal	parasyncolpate	m	
	n	<i>nitida</i>	70	coarsely reticulate	oblate spheroidal	6-colpate	m	
Polemoniaceae								
<i>Cobaea</i> (19)	b	<i>ascheronianiana</i>	170	reticulate	spheroidal	pantoporate	m	
	b	<i>scandens</i>	119	reticulate	spheroidal	pantoporate	m	53
	b	<i>trianae</i>	112	reticulate	spheroidal	pantoporate	m	56
	n	<i>lutea</i>	160	reticulate	spheroidal	pantoporate	m	77
	n	<i>penduliflora</i>	193	reticulate	spheroidal	pantoporate	m	118
Strelitziaceae								
<i>Phenakospermum</i> (1)	b	<i>guianense</i>	80	psilate (sparse microspinules)	spheroidal	inaperturate	m	
<i>Strelitzia</i> (5)	n	<i>reginae</i>	62	psilate with small spinules		inaperturate	m	
Velloziaceae								
<i>Barbacenia</i> (104)	b	<i>rubrovirens</i>	27	reticulate	prolate	monosulcate	m	
	n	<i>elegans</i>	22	reticulate	prolate	disulcate	m	

consequence of selection by current pollinators) instead. This would be in accordance with a less neat correlation. Herrera (1996) found that floral traits are often exaptations. Spatio-temporal unpredictability in the composition of pollinator assemblages is probably one of the most important factors reducing the possibilities of selection on floral traits by pollinators. Heterogeneous pollinator assemblages, as found for many plant species, also seriously weaken directional selection. Stebbins' most effective pollinator principle (Stebbins 1970) states that the characteristics of the flower will be moulded by those pollinators that visit it most frequently and effectively in the region where they are evolving. However, often the visitors providing the highest efficiency in pollen transport from anther to stigma are neither the most abundant nor the most predictable in time and space, thereby limiting the possibilities of selection on floral traits (Herrera 1996). But since the bat pollinated plants in this study were selected based on their adapted morphology it seems more plausible that in an evolutionary sense exine morphology is usually not a very flexible character but is heavily constrained. There seems to be no such thing as a pollen morphological component of the bat pollination syndrome. Future studies should therefore address parameters that deal more directly with pollinator-flower interactions in the field, like differences in amount of pollenkitt and stickiness.

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The structure and function of orchid pollinaria

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Abstract. Cohesive masses of pollen known as pollinia have evolved independently in two plant families – Orchidaceae and Asclepiadaceae. Yet, the bilateral symmetry of orchids has allowed a greater degree of specialization in pollination systems and a much greater diversity in the morphology of pollinaria – units comprising the pollinia(um) together with accessory structures for attachment to the pollinator. Pollinaria differ in the degree of cohesion of pollen in the pollinium, which may be soft, sectile (comprised of sub-units known as massulae) or hard. A single hard pollinium may contain more than a million pollen grains, yet pollen:ovule ratios in orchids are several orders of magnitude lower than in plants with powdery pollen due to the lack of wastage during transport to the stigma. Attachment of pollinia to the pollinator is usually achieved by means of a viscidium that adheres most effectively to smooth surfaces, such as the eyes and mouthparts of insects and beaks of birds. The stalk connecting a pollinium to the viscidium may be comprised of a caudicle (sporogenous in origin) and/or a stipe (derived from vegetative tissue), or be lacking altogether. Caudicles and stipes may undergo a gradual bending movement 20 s to several hours after withdrawal from the flower, the main function of which appears to be to reduce the possibility of geitonogamous pollination. Other mechanisms that promote outcrossing and pollen export in orchids include pollen carryover (achieved by sectile or soft pollinia), temporary retention of the anther cap, protandry and self-incompatibility (rare among orchids). Pollinaria ensure that large pollen loads

are deposited on the stigma, thus enabling the fertilization of the large numbers of ovules in the flowers of Orchidaceae. Pollinaria also ensure efficient removal of pollen from the anther, minimal pollen wastage during transit, and a high probability of deposition on conspecific stigmas.

Key words: Orchidaceae, geitonogamy, gene flow, pollen, pollen discounting, pollen:ovule ratio, pollinaria, pollination, pollinium, seed size, self-incompatibility.

The packaging of pollen into a compact unit known as the *pollinium*, which together with accessory structures for attachment to pollinators comprises a *pollinaria*, was undoubtedly a key innovation in the evolutionary history of the Orchidaceae, and may have played a role in promoting the tremendous radiation of the group, which numbers at least 19 500 species (Dressler 1993). The purpose of this review is to provide a synthesis of published information on structural and functional aspects of orchid pollinaria. In particular, we attempted to combine two different, yet complementary, traditions in the study of orchids, namely the comparative morphological approach of systematic botany (Dressler 1993) and the ecological approach adopted in experimental pollination biology (Nilsson 1992).

The structure and function of orchid pollinaria cannot be understood in isolation from

other profound morphological modifications that occurred during the evolution of the Orchidaceae. Among these were the reduction in the number of fertile stamens to just one, varying degrees of fusion of stamen and pistil into a structure known as the column (or gynostemium), and the dust-like seeds, most of which lack endosperm (Dressler 1981).

The adnation of the gynoecial and androecial whorls of the Orchidaceae and Asclepiadaceae sets these families apart in the Monocotyledonae and Dicotyledonae, respectively. In both families, pollen has been consolidated into pollinia. Both families show precursory evidence of grouped pollen grains in the form of tetrads, almost as if there has been repeated consolidation of the pollen loads during their evolution; however, the retention of actinomorphic flowers in the Asclepiadaceae has precluded the reduction in anther number that is so evident in the orchids.

The orchid anther

Hypothetical ancestral patterns of the orchids are based on a hexastaminate lilioid template (Dahlgren et al. 1985, Rasmussen 1986b, Cam-

eron et al. 1999) and require losses of 3, 4 and 5 functional anthers to produce the subfamilies Apostasioideae, Cypripedioideae and the monandrous orchids respectively (Table 1).

The Apostasioideae, with their primitively triandrous flowers and fairly poor fusion between the androecium and gynoecium, clearly represent the plesiomorphic condition among orchids (Dressler 1993). However the specialization of their vessels (Judd et al. 1993) indicates vegetative specialization off the main phyletic lineage. The monophyly of the subfamily is well established by recent studies (Judd et al. 1993, Stern et al. 1993, Freudentstein and Rasmussen 1999, Cameron et al. 1999). Diandrous species of *Apostasia* are linked to the triandrous pattern through members of the typical section which sport a median staminode, the loss of which is considered specialized (Dressler 1990). Anther dehiscence is introse via longitudinal slits. The anthers are dorso-basally attached and, while considerable connation of staminal filaments occurs, the style is largely free. Endothelial thickenings are intimately involved in anther dehiscence (Dahlgren et al. 1985) and in the Apostasioideae are tightly packed channels

Table 1. Some androecial characters of the Orchidaceae (*o* outer, *i* inner, *l* lateral, *m* median)

Taxon	Stamens	Staminodes	Pollinia	Viscidia
Liliaceae s.l.	6(3 <i>o</i> , 3 <i>i</i>)	0	absent	absent
Apostasioideae			absent	absent
<i>Neuwiedia</i>	3 (1 <i>om</i> , 2 <i>il</i>)	0		
<i>Apostasia</i>	2 (<i>i</i>)	0–1 (<i>om</i>)		
Cypripedioideae	2 (<i>il</i>)	1 (<i>om</i>)	smear pollen, rarely soft pollinia	absent
Spiranthoideae	1 (<i>om</i>)	2 (<i>il</i>)	soft pollinia, sometimes sectile	apical when present
Orchidoideae	1 (<i>om</i>)	2 (<i>il</i>)	soft pollinia or sectile with hard massulae	basal when present
Epidendroideae	1 (<i>om</i>)	2 (<i>il</i>)	hard or, rarely, soft pollinia	basal when present

of loops or helices. Similar patterns occur in putatively basal genera in the remaining sub-families (Freudenstein 1991). The reduced thickenings associated with the more "advanced" tribes may be due to the decrease in conventional anther dehiscence associated with the evolution of pollinaria and detachable anther caps.

The Cypripedioideae are diandrous and are further characterized by their prominent median staminodes, saccate lips and synsepalum. The group is monophyletic and is well supported in recent cladistic studies (Albert 1994, Cameron et al. 1999, Freudenstein and Rasmussen 1999). Their androecial derivation is from the loss of three adaxial stamens and retention of the lateral anthers as fertile units. The fertile anthers are subsessile and are positioned so that pollinators escaping from the labellum pass the convex stigma before coming into contact with them. The endothecial thickenings of this group are a circular arrangement of anticlinal bars which are convergent with patterns in the Vanillinae and Cranichidae (Freudenstein 1991). The median staminode is highly variable and is probably intimately involved in pollination syndromes. The organ has considerable systematic importance due to its divergence at the species level (Cribb 1987).

Within the monandrous Orchidaceae only the median abaxial anther is fertile and this is frequently flanked by the sterile remnants of the lateral abaxial stamens. With the reduction of fertile anthers comes a trend of increased pollen aggregation from primitive powdery or mealy pollen masses to complex pollinaria (Burns-Balogh and Funk 1986, Dressler 1986). In a few anomalous instances this typical pattern of androecial reduction is contradicted, thus *Encyclia cochleata* (L.) Lemee var. *triandra* has two supernumerary fertile anthers which are involved in autogamy (Catling 1990) and this situation is also recorded from *Epidendrum nocturnum* Jacq. (Ackerman 1995). Unusual androecial patterns are also present in *Diplandrochis* [= *Neottia*] which is unique in the occurrence of two fertile anthers

(the medial members of the inner and outer whorls of the androecium) and the Chinese genus *Tangtsinia* [= *Cephalanthera*] which has five staminodes and a single outer median stamen (Chen 1982).

Pollen

Possibly no other family of Monocotyledonae exhibits the range of pollen wall diversity apparent in the Orchidaceae. Pollen surface sculpturing is most complex in *Diseae* and *Orchidoideae*, which have sectile pollinia, and least complex in orchids with hard pollinia (Schill and Pfeifer 1977). Burns-Balogh (1983) suggested phylogenetic pathways for the evolution of the exine in orchids and a more extensive structural account is available in Schill and Pfeiffer (1977). Pollen grains within the Apostasioideae and Cypripedioideae, are loosely aggregated monads, while in the monandrous orchids, monads are largely replaced by tetrads and these may be loosely packaged or tightly fused into pollinia.

The pollen of the Apostasioideae is produced in monads and grains are monosulcate, operculate and have reticulate sculpturing (Newton and Williams 1978, Schill 1978). Examination of the pollen walls reveals a semi-tectate arrangement with a tectum, columellae and a foot layer. The operculum appears to be synapomorphic for this group (Judd et al. 1993, Freudenstein and Rasmussen 1999), although Burns-Balogh and Funk (1986) argued that the condition was derived separately in *Apostasia* and *Neuwiedia*. The progressive aggregation of pollen in the remaining orchids obviates the advantages of operculate pollen. Pollen sculpturing in the Apostasiaceae is reticulate and similar to that of the *Neottieae* (Williams and Broome 1976).

Throughout the Cypripedioideae pollen is produced as adherent monads, which smear insects escaping from the trap flowers. Pollinia occur in *Phragmipedium* and *Selenipedium* but these remain soft masses representing the contents of the four pollen sacs. TEM shows that the wall structure of cypripedioid pollen is

very distinctive. The foot layer has been lost and the imperforate tectum is accompanied by incipient columellae, a condition which is unique to this subfamily (Burns-Balogh 1983). In addition the sculpturing of the grains is smooth (Williams and Broome 1976, Newton and Williams 1978) which has been interpreted as a derived condition (Freudenstein and Rasmussen 1999).

Dressler (1981, 1993) subdivides the monandrous orchids into three subfamilies: Spiranthoideae, Orchidoideae and Epidandroideae. Basic pollen subunits are tetrads in all of these subfamilies; however, a number of taxa produce monads. Within the vanilloid orchids monad pollen grains are ubiquitous in the subtribes Vanillinae and Lecanorchidinae, and in the subtribe Pogoniinae, *Pogonia* and *Cleistes* have monads. *Palmorchis* (Palmorchidaceae) also has monads and was included in the Vanilleae by Dressler (1981) and considered vanilloid by Freudenstein and Rasmussen (1999). Recent molecular studies (Cameron et al. 1999) place *Palmorchis* as a sister group to the Neottieae where monads occur in *Cephalanthera* (subtribe Limodorinae). Recent evidence indicates that the vanilloid orchids are a fairly basal lineage in the Orchidaceae (Cameron et al. 1999), therefore monads in this group are not necessarily derived from tetrads.

Within the Orchidoideae both monads and tetrads have been recorded from the Diurideae in *Thelymitra* (subtribe Thelymitrinae), *Caladenia*, *Spiculaea* and *Chiloglottis* (subtribe Caladeniinae), *Codonorchis* (subtribe Chloraeinae), *Corybas* (subtribe Acianthinae), *Epiblema* and *Calochilus* (subtribe Diuridinae) and *Pterostylis* (subtribe Pterostylidinae). In *Caladenia patersoni* R. Br. both monads and tetrads were reported from the same sample. Thus some variability occurs in the pollen units of the Diurideae and Ackerman and Williams (1981) suggest that production of monads or tetrads may differ between populations or be influenced by environmental conditions. Careful consideration needs to be given to this question as it points to the secondary derivation of monads for some of these species.

The taxonomic position of some of the tribes with monads is fairly unstable (Ackerman and Williams 1980, 1981; Dressler 1981, 1993; Kores et al. 1997; Cameron et al. 1999; Freudenstein and Rasmussen 1999). Dressler (1986) cautions that slight ontogenetic changes may be responsible for the production of monads or tetrads and that monandrous orchids with monads are not necessarily primitive.

The exine development in orchid pollen grains depends, to some extent, on characteristics of the pollinium. In species with mealy pollinia all the grains usually have an exine (Fitzgerald et al. 1994) and cohesion is achieved by autolysis of the tapetum which releases a lipid glue. This substance eventually penetrates the entire pollinium and consolidates all the microspores (Fitzgerald et al. 1993). The early release of the consolidating lipid may be necessary to achieve complete coverage of all grains. By contrast in *Dendrobium* where grains are tightly consolidated the inner grains often lack exine layers (Zavada 1990). Similarly in *Epidendrum ibaguense* H.B.K. the mature pollen grains reveal layering. The exine is clearly separable into an amorphous nexine, which covers all grains, and the sexine which is concentrated on the periphery of the pollinium (Blackman and Yeung 1983). Consolidation of the pollinia is achieved by a secretion fairly late in pollinium development. Again, the adhesive material is produced in tapetal cells, but penetration is limited to the spaces between adjacent peripheral tetrads. On exposure to light and drying this layer polymerizes into a hard coat (Fitzgerald et al. 1994).

In sectile pollinia, such as those of *Loroglossum hircinum* (L.) C. Rich., pollen grains are grouped in tetrads which coalesce into massulae and again the exine is limited to peripheral grains. The intine of these species often comprises two layers; the outer layer maintains the integrity of the tetrad and the inner layer surrounds each grain (Pandolfi and Pacini 1995). In the Disinae pollen tetrads are calymmate, but the massulae and pollinia are acalymmate (Chesselet and Linder 1993).

The cohesion of pollen into pollinia must be constrained, to some extent, by the interference caused by pollen walls during germination. The reduction in exine surrounding inner grains may be part of the evolutionary compromise in reducing this interference. In addition agglutination of pollinia with substances which degenerate upon contact with the stigma alleviates constraints placed upon innermost tetrads. Pandolfi and Pacini (1995) indicated that intercellular spaces between microspores are dynamic in massulae and that they increase from 15–20% to 50–60% during germination. In addition changes may occur in the male and female gametophytes of orchids which lead to delayed and asynchronous emission of pollen tubes (Pacini and Franchi 1996). Pollinium morphology is, presumably, also mediated to facilitate germination (Pacini and Franchi 1998) and this may have favoured flattened pollinia and the preservation of longitudinal furrows between fused pollinia (Figs. 11, 12).

Pollinarium

In the majority of orchids, the pollinia are joined to an adhesive viscidium (Figs. 2, 4, 8, 9, 11, 12), forming a structure known as a pollinarium. The pollinarium is thus the entire structure, including one or more pollinia, that is removed by a pollinator. In the Orchidoideae and some Vandaeae where each of two pollinia are joined to separate viscidia (Figs. 9, 13), Dressler (1981) sought to introduce the term “hemipollinarium” on the technical ground that one anther can produce only one pollinarium. However, this term has not really been widely adopted, and in accordance with Dressler (1990), we use the term pollinarium to refer to the package comprising pollinia plus viscidium, regardless of whether there are one or two such packages in a flower.

The tremendous diversity of pollinaria, reflects the pivotal role of this structure in the complex pollination mechanisms of orchids, and has been found to be useful as a source of characters for taxonomy (Burns-

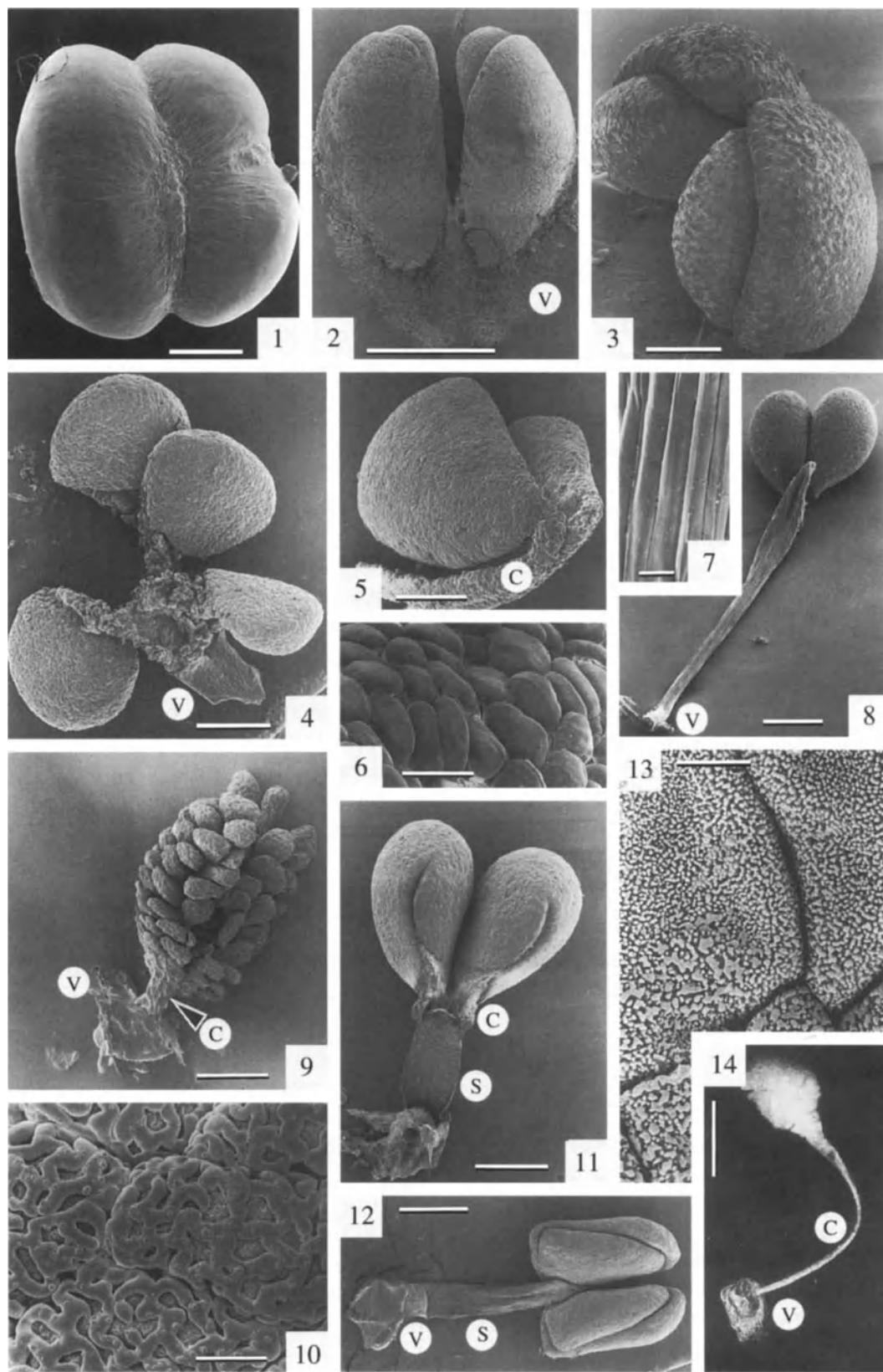
Balogh 1982, Ackerman and Williams 1981, Chase 1987).

Pollinia

The term pollinium refers to a more or less compact and coherent mass of pollen, a condition found in the great majority of orchids (Dressler 1993). The subdivision of the pollen within an anther is based on a pattern of four locules, each producing pollen masses. The production of four pollinia is thus plesiomorphic for many groups, though Dressler (1986) indicates that eight pollinia are produced in primitive members of the Arethuseae, Epidendreae and Dendrobieae. The Epidendreae show a reduction series of six, four (Fig. 4) and finally two pollinia while four pollinia occur in most derived genera of Dendrobieae. Rasmussen (1986a) argued that the plesiomorphic condition in the *Dendrobiiinae* and *Bulbophylinae* is more likely to be four.

Freudenstein and Rasmussen (1996) provided an ontogenetic model explaining differences in the numbers of pollinia among orchids. Sporogenous tissue is produced by a single meristematic region in each pollen sac and the number, shape and type of pollinia are produced by septation or fusion of these meristematic regions. Within the Epidendroideae septation of the meristem results in four or eight pollinia per anther while lack of septation gives two pollinia, common in many vandoid species (Dressler 1986). In contrast, the two bipartite pollinia found in many Spiranthoideae and Orchidoideae are produced by adherence of the contents of two locules at a late ontogenetic stage, and should be recognized as distinct. Within the latter subfamilies eight pollinia result either from partitioning of the meristem by two longitudinal septa or a longitudinal and a transverse septum (Freudenstein and Rasmussen 1996).

The clear elastic material that forms a matrix which binds pollen together within the pollinium, is known as elastoviscin (Dressler 1981). Elastoviscin in *Epidendrum* was reported to be a lipid polymer by Blackman and



Yeung (1983). Schill and Wolter (1986) consider elastoviscin to be homologous with pollenkit. A different material, termed cohesion strands, was found to bind the loose pollinia of some Spiranthinae by Burns-Balogh (1982). In the Disinae, additional pollen cohesion is established by means of tectal bridges (Chesselet and Linder 1993), while Ackerman and Williams (1981) reported unique, strap-like bands connecting grains within tetrads of the Diurideae. Schill and Wolter (1986) outline the ontogeny of elastoviscin in the family.

The cohesion of pollen grains is highly variable within the Orchidaceae. Loose, powdery or sticky pollen is confined to the Apost-

tasioideae and some Cypripedioideae, the remaining orchids having soft, sectile or hard pollinia, in order of increasing cohesion (Dressler 1986). Soft pollinia occur in *Selenipedium* and *Phragmipedium*, as well as in some of the monandrous orchids, such as *Vanilla*. The distinction between orchids having loose pollen masses and soft pollinia is not always clear, and we suggest that the term pollinia be used for any pollen masses that are removed in their entirety from the anther during a single pollinator visit.

Sectile pollinia are those that are subdivided into discrete units or massulae (Figs. 9, 13). The functional consequence, elaborated on in the second half of this review, is that several flowers may be pollinated from a single pollinium. Sectile pollinia have originated several times in the Orchidaceae, but can be grouped into two basic types. The first is restricted primarily to the Spiranthoideae and Orchidoideae and comprises fairly homogeneous wedge-shaped massulae attached, in a single layer, around an elongate core of elastoviscin (Freudenstein and Rasmussen 1997). The second type occurs within the Epidendroideae and comprises irregular massulae which are interconnected by weak strands of elastoviscin (Freudenstein and Rasmussen 1997). It is clear that the evolution of pollinia is closely linked with the evolution of the stigmatic surfaces upon which they are deposited. In the taxa with sectile pollinia the stigmatic surfaces are often flat or slightly convex and have a fairly shallow adhesive covering. This facilitates the gradual erosion and enhances pollen carryover (Nilsson 1983).

Hard pollinia, the kind most common among orchids, represent the greatest degree of cohesion of pollen, and are deposited as an entire unit on the stigma, in which respect they are similar to the pollinia of Asclepiadaceae. Pollinia which require deposition in a single event correspond to concave stigmatic surfaces with deep stigmatic fluid and sometimes modifications to the rostellum (Burns-Balogh and Bernhardt 1985). Some hard pollinia are waxy in composition, while Dressler (1993) describes

Figs. 1–14. Anther and pollinarium morphology. **Fig. 1.** Anther of *Paphiopedilum callosum* Pfitz. (Cypripedioideae). Bar: 400 µm. – **Fig. 2.** Pollinarium of *Coelogyne barbata* Lindl. ex Griff. (Epidendroideae). Bar: 1 mm. – **Fig. 3.** Sessile pollinarium of *Maxillaria variabilis* Batem. ex Lindl. (Epidendroideae). Bar: 200 µm. – **Fig. 4.** Pollinarium of *Epidendrum porpax* Reichb. f. (Epidendroideae) with four pollinia and well developed caudicles. Bar: 250 µm. – **Fig. 5.** Pollinia of *Cattleya deckeri* Klotzsch (Epidendroideae) with caudicles derived from modified pollinia. Bar: 200 µm. – **Fig. 6.** Cellular detail of caudicle tissue of *C. deckeri* showing tetrads. Bar: 20 µm. – **Fig. 7.** Cellular detail of the stipe of *Oncidium ornithorhynchum* H. B. and K. (Epidendroideae). Bar: 20 µm. – **Fig. 8.** Pollinarium of *O. ornithorhynchum* showing the well developed stipe and two terminal pollinia. Bar: 430 µm. – **Fig. 9.** Sectile pollinarium of *Stenoglottis longifolium* Hook. f. (Orchidoideae) with a discrete viscidium. Bar: 500 µm. – **Fig. 10.** Tectal sculpturing of pollen grains of *S. longifolium*. Bar: 5 µm. – **Fig. 11.** Pollinarium of *Gomesa recurva* Lodd. (Epidendroideae) with well developed stipe, reduced caudicles and two terminal pollinia each with a longitudinal cleft. Bar: 300 µm. – **Fig. 12.** Pollinarium of *Lycaste lasioglossa* Reichb.f. (Epidendroideae) with a well developed stipe and two lateral pollinia derived from 4 partially separated meristems. Bar: 600 µm. – **Fig. 13.** Tectal sculpturing of *Bonatea cassidea* Sond. (Orchidoideae). Bar: 9 µm. – **Fig. 14.** Pollinarium of *B. cassidea* showing the discrete viscidium and elongated sterile caudicle. Bar: 4 mm. Abbreviations: C caudicle; S stipe; V viscidium

the very hard pollinia of "advanced" Epidendroideae as "bony".

According to Dressler (1993), 80% of orchids have hard pollinia, 11% have sectile pollinia (mainly Orchidaceae and Deseae), 6.6% have soft pollinia (mainly Cramchideae and most Diuridae) and less than 3% have various types of powdery pollen/loose pollinia (exemplified by Apostasioideae, Cypripedioideae and Vanilleae).

Individual pollinia can contain between 5000 and 4 000 000 pollen grains (Schill et al. 1992). The fewest pollen grains recorded per pollinium are found in neottiod orchids, such as *Cephalanthera longifolia* Fritsch with 5547 grains (Nazarov and Gerlach 1997), intermediate numbers are found in sectile pollinia, such as those of *Orchis* species which vary between 36 000 and 62 000 grains (Darwin 1877, Nazarov and Gerlach 1997), and the highest numbers in orchids with solid pollinia, such as *Coryanthes senghasiana* G. Gerlach with 400 000 grains (Nazarov and Gerlach 1997).

Caudicles

In the monandrous orchids the adhesive compounds which attach pollinia to pollinators are derived from stigmatic tissues and this dictates a close proximity of the sexual parts. The evolution of such sexual integration carries with it the possibility of autogamy and a phylogenetic 'dead end'. Thus the sterility of the structures which connect the viscidia (stigmatic) with the pollinia must have been of cardinal importance in the family's divergence. In the Dendrobiinae, Bulbophylinae, Triphoreae (*Triphora*), Arethuseae (*Bletilla*) and Malaxideae (*Liparis*) there are no physical structures connecting the viscidial glue to the untailed pollinia but in most other groups pollinia abound with unusual artifices, derived from female tissues, which are instrumental in pollination success. In view of the proximity of sexual parts, which allow this integration, it is not surprising that autogamy occurs in 5–20% of orchid species (Catling 1990).

Caudicles are produced within the anthers and can be considered an extension of the pollinium. Their anatomical structure is consistent with derivation from sporogenous tissues (Van der Pijl and Dodson 1966, Blackman and Yeung 1983) making them a unique example of haploid tissues which have a nonsexual mechanistic function. Caudicles are a mixture of tetrads, aborted pollen mother cells and tapetal remnants in a matrix of elastoviscin. Occasionally they are devoid of pollen tetrads and comprise almost pure elastoviscin, e.g. *Lockhartia* and *Cryptarrhena* (Dressler 1986). In other instances whole pollinia serve as caudicles and these may be hard and hyaline, e.g. Podochilinae (Dressler 1981) or poorly differentiated in genera such as *Cattleya* (Fig. 5), *Epidendrum* and *Encyclia* (Fig. 4). The latter examples substantiate Dressler's (1981) assertion that 'the primitive pollinium number in the Epidendreae is eight'. The retention of eight pollinia in genera such as *Sophronitis*, *Ceratostylis*, *Brassovola* and *Laelia* would thus represent an unspecialized condition. This argument conforms with the notion that specialization of pollination syndromes often correlates with increased levels of pollen cohesion.

In the Spiranthoideae and Epidandroideae caudicles are connected to the apices of pollinia (acrotonic attachment) but in the Orchidoideae and a few other exceptions attachment is basal (basitonic attachment) (Freudenstein and Rasmussen 1999). Sterile caudicles in the sectile pollinia of the Orchidoideae are considered to have been derived from homogenous pollinia (Dressler 1993) (Fig. 9).

The remarkable elasticity of the caudicle appears to be due to the same viscin material that binds pollen tetrads in sectile pollinia, but we are not aware of any elucidation of the structure of this material. Caudicles may be up to 20 mm in length in some Habenariinae, such as *Cynorkis uniflora* Lindl. (Nilsson et al. 1992a) and *Bonatea speciosa* Willd. (Johnson and Liltved 1997) (Fig. 13). On the other hand, caudicles are reduced and hidden in a slit in some vandoid orchids

(Dressler 1993). In *Dendrobium*, hard pollinia are attached directly to the pollinator by means of a smear of glue from the rostellum (Dressler 1981).

After removal from the anther, many pollinaria undergo a bending movement localized at some point along the caudicle. Darwin (1877) showed that these movements are hygrometric and that after bending the pollinaria can be restored to its original position by exposure to water. Darwin (1877) showed that bending of pollinaria of Orchideae, including *Gymnadenia conopsea* and *Orchis mascula*, is localized at the point where the caudicle is joined to the viscidium. He suggested that it was the contraction of cells forming part of the viscidium that caused the bending movement to take place.

The caudicle usually serves as the breakage point between pollinia and viscidia during pollination. However, in orchids with sectile pollinia, such as the Orchideae and Diseaseae, the caudicle does not break and individual massulae, rather than whole pollinia, are deposited onto the stigmas.

Stipe

Caudicles are sometimes augmented by stalks, derived from non-sporogenous tissues, which are collectively termed stipes (Rasmussen 1986a). According to Rasmussen (1986a), the stipe is attached to the caudicle by means of viscid material from the anther and this often serves as a break point during deposition of the pollinium.

Where stipes are produced from the abaxial epidermis of the column and form vitreous plates they are referred to as tegulae (sing. tegula) (Rasmussen 1986a). Dressler's (1993) definition is broader, including all stipes derived from column tissue. Rasmussen (1986b) cautioned that the stipes present in the *Stanhopea* (Stanhopeinae) may not be homologous structures due to their multilayered nature; however, later developmental studies in *Gongora* and *Cirrhaea* show that the layers are produced by periclinal divisions within the

epidermis and thus represent an elaboration of the tegula (Freudenstein and Rasmussen 1996). Rasmussen (1986a) records tegulae from Vandae (Vanda and Cleisostoma), Oncidiinae (*Oncidium*) and Goodyerinae (*Hetaeria* and *Zeuxine*). Freudenstein (1994) augmented this list with Calypsoeae (*Calypso* and *Yoania*) and Cymbidieae (*Govenia* and *Eulophia*).

In a few isolated cases stipes are produced by sharply recurved rostellar tips which are detachable; these are termed hamulae (sing. hamulus). These structures occur in Epidendroideae: Bulbophylinae (*Bulbophyllum*, *Monomeria*), Orchidoideae: Prasophyllinae (*Prasophyllum* and *Microtis*) and Spiranthoideae: Tropideae (*Tropidia*, *Corymborkis*) (Rasmussen 1982a, 1986a). In these instances the structure is clearly convergent. Further examples of hamulae are listed from *Amblectrum*, *Corallorrhiza*, *Cremastra* and *Oreorchis* (Corallorrhizinae) where Freudenstein (1994) reports a strong correlation between the development of the hamulus and outcrossing. In these instances the structure is probably synapomorphic.

Perhaps the most bizarre function of the stipe among orchids is its role in forcibly ejecting the pollinaria out of *Catasetum* and *Mormodes* flowers. This mechanism, first elucidated in painstaking detail by Darwin (1877), relies on the natural elasticity of the stipe which is "springloaded", until explosively released when a bee touches a sensitive trigger. The pollinaria may be thrown more than a metre from the flower (Darwin 1877), and normally strikes a bee with sufficient force to cause subsequent aversive behaviour toward male *Catasetum* flowers, according to Romero and Nelson (1986).

It is clear from this brief synopsis that selection has driven considerable convergence and divergence in the evolution of stipes. Within the Oncidiinae, for example, stipes are highly divergent at the generic level (Williams 1972, Chase 1987). Pollinia stalks have also proved useful in a recent reassessment of the Corallorrhizinae (Freudenstein 1994).

Viscidium

In supposedly primitive orchids, such as the Cypripedioideae, an insect emerging from the flower receives a coating of stigmatic fluid on its back, to which pollen subsequently adheres as the insect brushes the anther. The role played by the stigma in pollen transfer has been refined in most orchids with the development of the viscidium, a sticky detachable structure derived from one of the stigmatic lobes that serves to attach the pollinia(ium) to the pollinator (Dresser 1993). The viscidium is connected to the pollinium by means of a caudicle, as well as a stipe in many orchids.

The sticky glue of the viscidium results from the breakdown of stigmatic cells and its composition remains a mystery, but it would be expected to be similar in structure to stigmatic mucilage. What is particularly interesting about the viscidium is the way the glue dries soon (usually within minutes) after coming into contact with the body of the pollinators. In some Orchidoideae the viscidium is covered with a macroscopic sheath (the bursicle) that is dislodged by the pollinator, triggering the drying process (Darwin 1877). An unusual mechanism of pollen deposition is found in the Listerinae which have a rostellum that squirts glue when touched; pollinia are released directly onto this fresh layer of glue (Ackerman and Mesler 1979). Viscidia of *Dendrobium* species burst on contact, leaving a smear of glue on the surface of the retreating pollinator, onto which the hard pollinia adhere (Rasmussen 1986b).

A single viscidium is found in most orchids, although many Orchidoideae, such as *Disa*, and some Vandaeae, have two viscidia associated with two pollinaria. These are usually removed at the same time, but in the case of twin-spurred *Satyrium* flowers and the functionally subdivided flowers of many *Habenaria* and *Bontaea* species, the viscidia are removed independently (Johnson 1997, Johnson and Liltved 1997). Within *Disaeae*, a single viscidium has been secondarily derived at least twice; in *Disa* section *Herschelia* by fusion of two

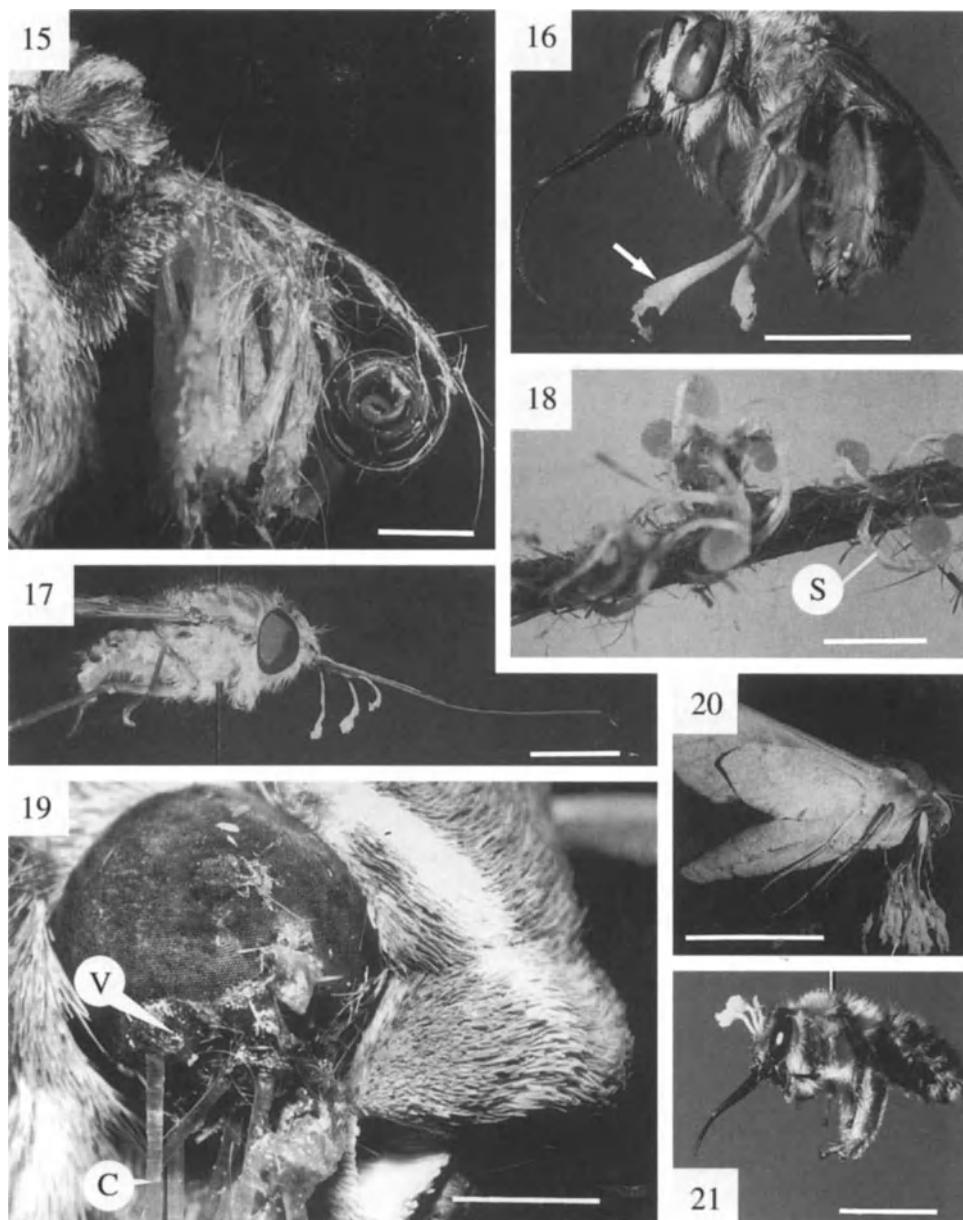
viscidia from the lateral rostellum lobes, and in *Disa* section *Monadenia* by the innovation of a single viscidium derived from an unlobed rostellum (Linder and Kurzweil 1994, Johnson et al. 1998a).

Pollen longevity in orchids

Orchids often occur at low densities and their pollinators seldom confine their visits to orchid flowers alone, thus it is likely that several days may sometimes elapse from when a pollinarium is removed to when it is eventually deposited on a stigma. Orchid pollen within intact pollinia would thus be expected to remain viable for relatively long periods of time. Sectile pollinia of *Dactylorhiza purpurella* (T. & T. P. Steph.) Soo retained their germinability for up to 51 days after removal from the flower and storage under laboratory conditions (Neiland and Wilcock 1995), while ageing of pollen for up to eight days in a laboratory had little effect on fruit formation in *Calopogon tuberosus*, *Pogonia ophioglossoides* and *Cypripedium reginae* Walt. (Proctor 1997). Field studies by Alexandersson (1999) showed that a labelled pollinium of *Calypso bulbosa* Reichb.f. was still capable of producing a normal fruit 10 days after being removed from a flower by a bumblebee. These studies indicate a great capacity for long-distance dispersal and infrequent pollination events among orchids.

Sites of pollinarium attachment

The powdery pollen typical of the majority of angiosperms adheres most effectively to the hairy parts of insects and mammals, and feathers of birds. By contrast, the viscidium of orchid pollinaria adheres most effectively to smooth surfaces that are generally unsuitable for the placement of powdery pollen (Figs. 15–21). This simple fact about pollen attachment helps to explain many of the morphological differences between the flowers of orchids and those of other families. Bird-pollinated orchids, for instance, tend to place pollinaria on



Figs. 15–21. Attachment of orchid pollinaria to insect pollinators. – **Fig. 15.** Pollinaria of *Disa ophrydea* Lindl. (Diseae) attached to the tongue of a moth, *Cucullia minuta* (Noctuidae). Bar: 1 mm. – **Fig. 16.** Sectile pollinaria of the orchid *Disa racemosa* L.f. (Diseae) adhere to the middle legs of the bee *Amegilla niveata* (Anthophoridae). Chunks of massulae have broken away from the arrowed pollinium. Bar: 5 mm. – **Fig. 17.** Long tongued fly *Prosoeca ganglbauri* (Nemestrinidae) with pollinaria of *Brownleea macroceras* Sond. (Diseae) attached to the proboscis. Bar: 5 mm. – **Fig. 18.** Close-up view of the proboscis of a hawkmoth showing attachment of hard-type pollinaria of *Mystacidium venosum* Harv. ex Rolfe (Aerangidinae). Bar: 5 mm. – **Fig. 19.** Eye of a hawkmoth, *Theretra capensis* (Sphingidae), showing attachment of pollinaria of *Bonatea speciosa* L.f. (Willd.) (Habenariinae). Bar: 2 mm. – **Fig. 20.** Hawkmoth, *T. capensis*, with a large load of *B. speciosa* pollinaria attached to its eyes. Bar: 20 mm. – **Fig. 21.** Pollinaria of *Satyrium erectum* Sw. (Satyriinae) attached to the frons of a bee, *Anthophora diversipes* (Anthophoridae). Bar: 10 mm. Abbreviations: C caudicle; V viscidium; S stipe

the bird's beak and therefore have short floral tubes, as it is necessary for a bird to insert its beak only a short distance into the flower, in contrast to the long floral tubes of flowers which deposit pollen on the feathers of a bird's head (Johnson 1996). Dressler (1981) suggested that the absence of bat pollination in orchids was due to the lack of suitable surfaces for pollinaria attachment on the bodies of these hairy mammals.

The precise placement of pollinaria on the bodies of pollinators, for which orchids are renowned, is achieved, firstly by filtering floral visitors by specific visual and olfactory cues, and secondly by modifications of the column and perianth parts that ensure that the pollinator is manipulated into the correct position for contact to be made between the viscidium and the exact target area on its body (Dressler 1981).

Pollen deposition in the vast majority of orchids is nototrophic (on the dorsal surface of the pollinator), but sternotrophic pollen deposition (on the ventral surface) does occur in the Diseaseae, and some Habenariinae and Diuridinae (Johnson et al. 1998b, Burns-Balogh and Bernhardt 1988). Commonly used sites for pollinaria attachment to insects include their mouthparts, frons, eyes, thorax, and legs (Figs. 15–21). Dressler (1981) identified 13 discrete sites for pollinaria placement on the bodies of euglossine bees. Rasmussen (1986b) describes how pollinia of *Dendrobium infundibulum* Lindley are attached precisely to a bald spot on the metanotum of the bumblebee pollinator.

A factor which undoubtedly influences the site of pollinaria placement is the probability that pollinators will remove the pollinaria. Bees, for example, frequently attempt to remove pollinaria, even when they are placed in such relatively inaccessible sites as the dorsal surface of the thorax (van der Pijl and Dodson 1966). Roubik (this volume) suggests that social meliponine bees may co-operate in the removal of pollinia from their bodies while in the nest, thus reducing the effectiveness of pollen export. Dressler (1971) made the inter-

esting suggestion that the dull colour of pollinaria of bird-pollinated species is an adaptation to render the pollinaria cryptic and therefore less likely to be scraped off the bill.

The size and shape of the viscidium is of tremendous importance for the success of pollen export in orchids. Viscidia range in size from the tiny viscidium of *Liparis capensis* Lindl. to the very large platelike viscidia of *Satyrium carneum* R. Br. that adhere firmly to the beaks of sunbirds (Johnson 1996). Viscidium shape varies from the long flat viscidium of *Spiranthes* which adheres to the flat and rigid galea of the proboscis of bee pollinators (Catling 1983) to the chunky viscidium of *Catasetum* which is forcibly propelled onto the thorax of visiting euglossine bees (Romero and Nelson 1986). Viscidia that attach to the narrow proboscides of Lepidoptera tend to encircle the tongue, as in *Anacamptis pyramidalis* Rich. (Darwin 1877) and *Satyrium stenopetalum* Lindl. (Johnson 1997). Eye-placement of pollinaria is quite widespread in the moth-pollinated Habenariinae, such as *Bonatea speciosa* (Figs. 19–20) (Johnson and Liltved 1997), *Platanthera chlorantha* Cust. ex Reichb. and *Cynorkis uniflora* (Nilsson et al. 1992a), and is also found in some Satyriinae with elongated rostellum arms and globose viscidia, such as the fly-pollinated *Satyrium bracteatum* Lindl. (Johnson 1997). Pollinaria of orchids pollinated by Lepidoptera are invariably attached to the eyes or proboscis (and rarely the legs – Johnson and Bond 1994), as these are the only sites that are not covered with scales (Figs. 15, 18–20).

Depending on the precision of pollinaria placement and the available space on the body of the pollinator, there may be restrictions in the number of pollinaria that can be attached to a single insect. For example, only one pollinaria of *Disa racemosa* Linn.f. can be attached at a time to a site on each of the middle legs of the anthophorid bees that pollinate this species (Fig. 16) (Johnson et al. 1998b). Only one pollinaria of *Catasetum pileatum* is usually found attached to the

euglossine bees that pollinate this orchid, but this has been attributed to aversion behaviour by the bees after experiencing the explosive mechanism of pollinarium attachment (Romero and Nelson 1986).

Pollen loads may become so large that insects are unable to fly properly, as observed for butterflies carrying *Disa uniflora* Berg. pollinaria (Johnson and Bond 1994) and hawkmoths carrying the formidable pollinaria of *Bonatea speciosa* (Fig. 20) (Johnson and Liltved 1997). Bernhardt (1995) suggested that *Pterostylis* releases the contents of one anther sac at a time, producing four discrete pollinia, to avoid overburdening the tiny mycetophilid gnats that pollinate these orchids. The impact of large orchid pollinarium loads on the proboscis and eyes of pollinators has not been investigated, but studies of Asclepiadaceae have shown that large numbers of pollinia attached to the feet and mouthparts of bumblebees lead to a significant decline in foraging efficiency (Morse 1981).

Several sympatric orchids may share a pollinator without competitive interference in pollen deposition due to the precision of pollinarium placement on different parts of the body of the pollinator (Dressler 1968, Nilsson 1983b, Steiner 1989). Switches from tongue to eye-placement appear to have been important

for speciation in *Platanthera*; at least three species pairs in the genus are differentiated primarily by placement of the pollinaria (Hapeman and Inoue 1997). The present-day distributions of these species pairs are at least partly overlapping, but it is difficult to establish whether selection for isolating mechanisms played any role in the shifts in pollinarium placement.

Mechanisms of pollen deposition on stigmas

Pollen is deposited onto orchid stigmas either when whole pollinia become trapped in a rostellum notch and detach from the viscidium, or when the adhesion between pollen and stigmatic mucilage is powerful enough to cause whole pollinia, individual massulae or parts of soft pollen masses to break away from the remainder of the pollinarium attached to the insect.

The success of pollen deposition in orchids is dependent on a sophisticated system of breakage points. In orchids with hard pollinia, it is imperative that the adhesion between the stigma and pollinium, as well as between the viscidium and pollinator, is adequate to ensure that the pollinarium breaks at the correct point, which is usually between caudicle and stipe. The retention of the anther cap for up to

Table 2. Pollen:ovule ratios reported for orchids

Type of pollinum	Method of deposition	Genera studied	Mean pollen: ovule ratio	SD	range	n	Reference
Soft	Scraping	<i>Isotria</i> , <i>Cephalanthera</i>	3.3	0.4	2.9–3.9	6	Mehrhoff (1983), Nazarov and Gerlach (1997)
Soft	Contact	<i>Listera</i>	30.1	0	—	1	Nazarov and Gerlach (1997)
Sectile	Contact	<i>Orchis</i> , <i>Dactylorhiza</i> , <i>Ophrys</i> , <i>Platanthera</i>	19.1	7.3	10–36	19	Neiland and Wilcock (1995), Nazarov and Gerlach (1997)
Solid	Scraping	<i>Coryanthes</i>	1.2	0	—	1	Nazarov and Gerlach (1997)

40 minutes in *Tipularia discolor* was shown to prevent deposition of pollinia since the force of adhesion between the anther cap and stigma was not sufficient to break the pollinium away from the caudicle (Catling and Catling 1991).

In most orchids with soft or sectile pollinia, pollination occurs when chunks of pollen or massulae adhere to the stigma and break away from the rest of the the pollinarium (Fig. 16). Here it is essential that the weak link is between the massulae and the elastoviscin core of the pollinarium, as this type of pollinarium can serve to pollinate a sequence of flowers. The stretchiness of the elastoviscin ensures that an entire pollinarium in contact with the stigma is not jolted off the insect when it departs from the flower. The degree of pollen carryover in orchids with sectile pollinia would be in part determined by the degree of cohesion of the individual massulae by elastoviscin threads.

Pollen: ovule ratios

Cruden (1977 and this volume) presented compelling evidence that the ratio of pollen:ovule number in angiosperms was a reflection of the efficiency of the pollination process in terms of the probability of pollen grains reaching a stigma. Low pollen:ovule ratios are found in autogamous and cleistogamous species, while the highest ratios (mean of 5900:1) are found in xenogamous species. However, outbreeding plants in which pollen is clumped, either being held together by viscin threads (as in Onagraceae and Caesalpinoideae) or aggregated into pollinia (as in Asclepiadaceae and Orchidaceae) do not conform to this trend and have pollen:ovule ratios many times lower than would be expected (Cruden and Jensen 1979, Proctor and Harder 1994, Nazarov and Gerlach 1997).

Pollen:ovule ratios for orchids appear to vary according to the degree of pollen clumping and the method of pollen deposition, with the lowest ratios (1:2) being found in orchids with hard pollinia and scraping deposition, and the highest ratios (19:1) in orchids with

sectile pollinia with contact deposition. Orchids with soft pollinia vary from a ratio of 3:1 in species with scraping deposition and 30:1 in a *Listera* species with contact deposition (Table 3).

Variation in pollen:ovule ratios in orchids is likely to reflect pollen carryover and its influence on typical stigmatic pollen loads. The relatively high pollen:ovule ratios in orchids with sectile pollinia, for example, can be explained in terms of their extensive pollen carryover. A single pollinarium of *Orchis mascula* L. can pollinate at least eight flowers (Johnson and Nilsson 1999), and typical stigmatic loads of *Orchis* species are only about a quarter of the amount of pollen found in a pollinium (Neiland and Wilcock 1995). In orchids in which an entire solid pollinium is deposited on a stigma, it would be expected that the pollen:ovule ratio would be about one, as borne out Nazarov and Gerlach's (1997) study of *Coryanthes senghasiana* (Table 2). Orchids with soft pollinia have either a scraping type rostellum which presumably removes much of the pollen load and may explain the low pollen:ovule ratios in species of *Cephalanthera* and *Isotria* (Nazarov and Gerlach 1997, Mehrhoff 1983), or a contact deposition of the *Orchis* type which may allow more extensive pollen carryover and explain the high pollen:ovule ratio of 31:1 in *Listera ovata* R. Br. (Nazarov and Gerlach 1997).

The number of pollen grains per pollinium in orchids varies from as few as 5500 in *Cephalanthera longifolia* to as many as 400 000 in *Coryanthes senghasiana* (Nazarov and Gerlach 1997). The number of ovules in orchids flowers is correlated with the number of pollen grains typically deposited onto the stigma after a pollinator visit (Proctor and Harder 1994, Neiland and Wilcock 1995, Nazarov and Gerlach 1997).

Spatial dispersal of pollinaria

Gene flow via pollen and seeds is important for determining the spatial scale of genetic divergence within and among plant populations

Table 3. Published data on dispersal of pollinia within orchid populations

Species	Pollen vector	Pollinium type	Method	Geitonogamy (% of pollen transfers)	Mean pollen transfer distance (m)	Maximum pollen transfer distance	Reference
<i>Prasophyllum fimbria</i>	Bees and wasps	Sectile	Histochemical stain	22	8	27	Peakall (1989a)
<i>Caladenia tentaculata</i> #	Wasps	Sectile	Histochemical stain	<10	17	58	Peakall and Beattie (1996)
<i>Microtis parviflora</i>	Ants	Sectile	Histochemical stain	70	0.22	0.45	Peakall and Beattie (1991)
<i>Cypripedium calceolus</i>	Bees	Ssoft	Histochemical stain	0	5.2	23	Tremblay (1994)
<i>Comparertia falcata</i>	Humming bird	Solid	Histochemical stain	85	—	—	Salguero-Faria and Ackerman (1999)
<i>Aerangis ellisiae</i>	Hawk-moth	Solid	Microtags	30	c. 5	76	Nilsson et al. (1992b)
<i>Calypso bulbosa</i> #	Bumblebee	Solid	Microtags	18–37	15	200	Alexandersson (1999)

single-flowered inflorescences

(Levin and Kerster 1974). Factors influencing pollen dispersal include behaviour of the pollinators, degree of pollen carryover from flower to flower and the density of the population (Levin 1981).

Studies of plants with loose pollen have shown that pollen dispersal curves are invariably leptokurtic with most pollen being dispersed among neighbouring plants a few meters away, and occasional dispersal to plants several hundred meters away (Table 4). Methods such as the use of dye analogues for loose pollen are generally unsuitable for measurement of dispersal of orchid pollinaria, necessitating the use of novel methods. These include the “microtags” developed by Nilsson et al. (1992b). Uniquely numbered tags with a breadth of only 0.5 mm are glued to individual pollinia while still in the anther, and subsequently traced to their deposition sites on stigmas, enabling the very accurate measurement of intra and inter-plant dispersal of pollinia. Results for the hawkmoth-pollinated

Malagasy orchid *Aerangis ellisiae* Schlechter show that most transfer occurred within 5 meters from the parent plant, with occasional dispersal as far as 76 m (Table 4). The frequency of geitonogamous pollen transfer was 30%. The same technique was used by Alexandersson (1999) to measure dispersal of pollinia of the boreal orchid *Calypso bulbosa* (Table 3).

Microtags are suitable only for orchids with solid-type pollinia. For studies of orchids with massulate pollinia, Peakall (1989a) developed a technique whereby pollen is coloured by histochemical stains applied in drop form to pollinia while still in the anther. Up to six colours can be used simultaneously within one population. Results for *Prasophyllum fimbria* Reichb.f. using this technique showed that 22% of pollen transfers were geitonogamous and that the mean pollen flow distance for allogamous pollen transfer was 8 m (Peakall 1989a). By contrast 51% of the pollen flow in the ant-pollinated orchid *Microtis parviflora* R.

Table 4. Recorded bending times for orchid pollinaria

Species	Location of bending	Bending time	n	Comments	Reference
<i>Dactylorhiza sambucina</i>	Caudicle	20 s	?	Generalized food-deception, bee-pollinated	Nilsson (1980)
<i>Orchis spitzelii</i>	Caudicle	26 s	20	Generalized food deception, bee-pollinated	Fritz (1990)
<i>Orchis morio</i>	Caudicle	30 s	25	Generalized food deception, bee-pollinated	Johnson and Nilsson (1999)
<i>Orchis mascula</i>	Caudicle	40 s	27	Generalized food deception, bee-pollinated	Johnson and Nilsson (1999)
<i>Himantoglossum hircinum</i>	caudicle	30 s	?	Deceptive, bee-pollinated	Darwin (1877)
<i>Orchis militaris</i>	Caudicle	42 s	30	Generalized food deception, bee-pollinated	Johnson and Nilsson (unpublished)
<i>Anacamptis pyramidalis</i>	Caudicle	30 s	10	Deceptive, lepidopteran-pollinated	Johnson and Nilsson (unpublished)
<i>Gymnadenia conopsea</i>	Caudicle	32 s	3	Nectariferous, lepidopteran-pollinated	Darwin (1877)
<i>Platanthera chlorantha</i>	Caudicle	2 s	30	Nectariferous, lepidopteran-pollinated	Johnson and Nilsson (unpublished)
<i>Platanthera blephariglottis</i>		30–60 s	21	Nectariferous, moth-pollinated	Darwin (1877)
<i>Coeloglossum viride</i>	Caudicle	80 s	?	Nectariferous, lepidopteran-pollinated	Johnson and Nilsson (1999)
<i>Ophrys insectifera</i>	Caudicle	60 s	?	Nectariferous, lepidopteran-pollinated	Cole and Firmage (1984)
<i>Coeloglossum viride</i>	Caudicle	6 min	3	Nectariferous, wasp- and beetle-pollinated	Johnson and Nilsson (unpublished)
<i>Ophrys insectifera</i>	Caudicle	20–30 min	?	Sexual-deception, wasp-pollinated	Darwin (1877)
<i>Cycnoches ventricosum</i>	Stipe	6 min	?	Sexual-deception, wasp-pollinated	Darwin (1877)
<i>Cycnoches lehmannii</i>	Stipe	15 min	?	Pollinated by male euglossine bees	Darwin (1877)
<i>Catasetum eburneum</i>	Stipe	40 min	?	Pollinated by male euglossine bees, anther cap falls off after 2–3 hrs	van der Pijl and Dodson (1966)
		“Several minutes”	?	Pollinated by male euglossine bees, hermaphrodite flowers	van der Pijl and Dodson (1966)

Table 4 (continued)

Species	Location of bending	Bending time	n	Comments	Reference
<i>Oncidium</i> sp.	Stipe	“Several hours”	?		Darwin (1877)
<i>Mormodes ignea</i>	Stipe	12–15 min	15	Pollinated by male euglossine bees	Darwin (1877)
<i>Mormodes luxata</i>	Stipe	15 min	?	Pollinated by male euglossine bees	Darwin (1877)
<i>Mormodes</i> aff. <i>buccinator</i>	Stipe	30 min	?	Pollinated by male euglossine bees	van der Pijl and Dodson (1966)

Br. were within the same inflorescence and the mean pollen flow distance was only 22.2 cm (Table 3). Observations of pollinator behaviour on orchids suggest that pollen dispersal distances may be greater in deceptive species due to the tendency of pollinators to depart from unrewarding patches, and conversely to remain in rewarding patches (Nilsson 1980, Peakall and Beattie 1996, Alexandersson 1999).

Mechanisms that promote outcrossing and efficiency of pollen transfer

Geitonogamy (transfer of pollen among flowers of the same plant), can reduce plant fitness in several ways: it can lead to inbreeding depression in self-compatible species and clog the stigma, and can also diminish the male component of reproductive success through pollen discounting (reduction in the efficiency of pollen export). For these reasons, it is not surprising that most orchids show sophisticated floral adaptations to maximize cross-mating opportunities.

1. Pollen carryover. Pollen carryover has not been well studied in orchids, partly due to the formidable difficulties in carrying out suitable experiments. It would be expected that pollen carryover would be limited in orchids with solid pollinaria, and be more extensive in orchids with sectile or mealy pollinaria.

Johnson and Nilsson (1999) allowed insects carrying freshly withdrawn pollinaria to visit a sequence of emasculated virgin flowers of

Orchis mascula and *Platanthera chlorantha* in a greenhouse. The average number of flowers receiving pollen from a single pollinium was 6.6 for *O. mascula* and 13.8 for *P. chlorantha*. The fraction of pollen carried over from flower to flower was 0.67 for *O. mascula* and 0.87 for *P. chlorantha*. This difference is probably due to the greater number of massulae within individual pollinia of *P. chlorantha* (366, versus 70 in *O. mascula*) and the gentler action of the moth pollinators of *P. chlorantha* compared to the bumblebees that pollinate *O. mascula* (Johnson and Nilsson 1999). Interestingly, these pollen carryover figures are within the range of 0.5–0.99 reported for plants with loose pollen (Robertson 1992 and refs therein), suggesting that at least for orchids with sectile pollinia, restricted pollen carryover may not pose serious problems for geitonogamy and pollen discounting.

While solid pollinaria would be expected to have no carryover, i.e. be deposited in the next flower that is visited, this is not the case in practice. The capture of insects carrying large loads of solid-type pollinaria indicates that pollen carryover may be extensive due to imperfections in the pollen-transfer process. In the case of solid-type pollinaria that first undergo a bending movement before insertion in the stigma, insects may visit a long sequence of flowers before pollination occurs.

In general pollen carryover is beneficial to the male component of plant fitness as it reduces the possibility of geitonogamy and increases the number of potential mates. One

way in which pollen carryover could be achieved in orchids with solid-type pollinia, is to have several pollinia in one pollinarium. Orchids which have up to eight solid-type pollinia in one pollinarium are particularly interesting as up to eight flowers in a sequence could theoretically be pollinated by a single pollinarium, although no evidence exists to validate this possibility.

2. Bending movements of pollinaria. After withdrawal from a flower, the caudicle or stipe of many orchids undergoes a bending movement such that the pollen masses become correctly orientated to strike the stigma. The movement may take anything from a few seconds to several hours (Table 4). The fact that the bending movement occurs slowly was interpreted by Darwin (1877) as a mechanism to prevent self-pollination among flowers on the same plant (geitonogamy). In some cases bending movements are involved in autogamy, as shown for example in the bee orchid *Ophrys apifera* Huds. (Darwin 1877, Catling 1990).

The degree to which gradual pollinarium bending will act to reduce or eliminate geitonogamy will depend on the time taken for a pollinator to leave an inflorescence. Observation of deceptive *Orchis* species in Europe indicate that pollinators spend less time on an inflorescence than the time taken for a pollinarium to undergo a bending movement, thus outcrossing is virtually assured (Nilsson 1980, Johnson and Nilsson 1999). In *Orchis mascula* L., for instance, pollinaria take an average of 30–40 s to undergo bending after withdrawal, yet bumblebee pollinators spend less than 10 s per inflorescence (Nilsson 1983a). Likewise with *Dactylorhiza sambucina* (L.) Soo, Nilsson (1980) found that bumblebees never spend more than 20 s on an inflorescence which is the time that it takes for pollinarium bending to occur in this species (Table 3).

Pollinators visit more flowers and forage longer on the inflorescences of nectariferous orchids. This is borne out by the observations of Dafni and Ivri (1979) who found that bees spend just 2–5 s on the deceptive flowers of *Orchis collina* Sol., as opposed to 10–60 s on

the nectar-producing inflorescences of *Orchis coriophora* L., and Johnson and Nilsson (1999) who found that addition of artificial nectar to flowers of *Orchis mascula* and *Orchis morio* L. increased the mean amount of time spent by bumblebees on an inflorescence from less than 10 s to about 60 s. As a consequence, it would be expected that natural selection would favour relatively delayed pollinaria bending times in nectariferous orchids. Data are sketchy, but indicate that pollinarium bending is effective in preventing geitonogamy even in nectariferous orchids. Pollinaria bending time for the rewarding orchid *Platanthera blephariglottis* Lindl. is about 60 s, longer than the c. 34 s that lepidopteran pollinators spend on average on an inflorescence (Cole and Firmage 1984). Pollinaria bending for *Platanthera chlorantha* takes 80 s, which is longer than the time taken by moths to visit an inflorescence (Johnson and Nilsson 1999).

Nierenberg (1972) suggested that bending of the stipe in *Oncidium* species was related to the amount of time spent by the pollinators, but unfortunately actual times for pollinator visits and bending of the stipe were not given. The longest pollinaria bending times (up to several hours) are found in orchids with stipes and solid type pollinaria, such as *Oncidium* (Table 4). This may partly be due to the fact that orchids with solid-type pollinaria lack pollen carryover, making them particularly vulnerable to geitonogamy when pollinators visit several flowers on an inflorescence. Euglossine bees are known to linger for long periods at fragrance-producing orchid flowers as they focus on fragrance collection. Dressler (1981) notes that euglossine bees will often re-enter the same flowers several times, thus posing a risk of self-pollination. For this reason, long pollinaria bending times would be expected in euglossine bee-pollinated orchids as a mechanism to prevent geitonogamy. Indeed, some of the longest pollinarium bending times are found in genera such as *Cycnoches* and *Mormodes* (Table 4). Pollinaria of *Mormodes* curl up after removal from the anther and gradually straighten out. However,

the elaborate pollinarium bending (technically straightening) found in *Mormodes* and *Cycnoches* is difficult to explain in terms of the outcrossing/reduction of pollen discounting hypothesis as these orchids largely have unisexual flowers.

3. Shrinking of the pollinium. It was recently reported that pollinia of *Bulbophyllum involutum* Borba, Semir and F. Barros and *B. ipanemense* Hoehne shrink in width by as much as 50% after removal from the anther, apparently as a result of dehydration (Borba and Semir 1999). Only after 105–135 minutes do the pollinia shrink enough to be inserted in the narrow entrance to the stigmatic cavity. Since the milichiid fly pollinators of these species spend up to about 15 minutes in a flower after removing the pollinaria, the mechanism appears to prevent self-pollination (Borba and Semir 1999).

4. Retention of the anther cap. Dressler (1981) suggested that retention of the anther cap for a few minutes to a few hours after a pollinarium is withdrawn from a flower would have much the same effect as pollinarium bending, namely to reduce the incidence of geitonogamous self-pollination. According to van der Pijl and Dodson (1966) anther caps of *Catasetum* species are retained for at least 20 minutes after ejection from the anther, and in *Cycnoches lehmanni* Nichols. for 2–3 hours after removal from the column. In *Tipularia discolor* the anther cap is retained for 8–40 minutes after pollinarium removal, the exact time being dependent on the ambient humidity (Catling and Catling 1991).

5. Protandry. In the majority of orchid flowers, removal and deposition of pollen happen simultaneously with little risk of self-pollination because pollinaria are removed only as the insect backs out of the flower, or, as discussed above, pollinaria have first to undergo a bending movement before they are in a position to contact the stigma. Nevertheless, protandry (male followed by a female phase in flowers) does occur in orchids (van der Pijl and Dodson 1966, Ackerman and Mesler 1979). The nectariferous flowers in

Spiranthes are protandrous (accomplished by changing the position of the column with respect to the lip) and open sequentially from the base of the tall upright inflorescence (Darwin 1877, Catling 1983). Foraging bumblebees land at the bottom of an inflorescence and forage upwards, thus ensuring that pollen is deposited on the older and lower flowers in the female stage and removed from the upper and younger flowers in the male stage – thus cross-pollination and pollen export is maximized. A similar strategy occurs in the related genus *Goodyera* (Ackerman 1975).

Protandry may also be effective in orchids which have a tendency to be revisited repeatedly by the same pollinator. Warford (1992) observed euglossine bees lingering for as long as 45 minutes at *Notylia* inflorescences. *Notylia* flowers are strongly protandrous, the stigma remaining tightly closed for two days after anthesis, and then opening to reveal a narrow slit just wide enough to accommodate one of the wafer-like pollinia (Warford 1992). Euglossine bee-pollinated orchids in the Catasetinae are either protandrous, as in some species of *Mormodes*, *Clowesia* and *Dressleria*, or unisexual, as in *Catasetum* and *Cycnoches* (Romero and Nelson 1990). These traits, together with the pollinarium bending and anther cap retention found in the group, may represent a suite of adaptations which ensure outcrossing and efficient pollen export despite the tendency of euglossine bees to linger and repeatedly enter flowers. Protandry also occurs in the Stanhopeinae, a subtribe in which pollination by euglossine bees is ubiquitous.

6. Self-incompatibility. Most orchids appear to be self-compatible, yet most of these appear to be predominately outcrossing as a result of mechanisms such as floral longevity, pollinarium bending and deception that reduce within-plant pollen transfer (geitonogamy). Even in self-compatible orchids, reduction in seed set is generally in evidence following self-pollination (cf Nilsson 1983a, Johnson 1994). This may be a consequence of inbreeding depression due to expression of deleterious alleles previously masked by heterozygosity.

Self-incompatibility appears to be rare among orchids but does occur in *Coelogyne*, *Lycaste*, *Chondrorhyncha* (Edwards unpublished data), *Notylia* (Warford 1992), *Oncidium* (Scott 1865, Sanford 1964) and *Dendrobium* (Johansen 1990). Self-incompatibility in orchids appears to be gametophytic and is usually expressed in flower abscission and not inhibition of germination or inhibition of pollen tube growth (Johansen 1990).

It is notable that in many of the orchid genera known to be self-incompatible, individuals are characterized by mass flowering which increases the possibility of geitonogamy. Self-pollinated flowers of many Epidendroideae fall off the plant, but in most *Orchidaceae* and *Diseae* self-pollinated flowers develop into fruits, albeit with fewer seeds than outcrossed flowers. In some orchids self-pollination results in fruits that are indistinguishable from outcrossed fruits in terms of seed number (Peakall 1989b) or seed fertility (Ackerman and Montalvo 1990, Salguero-Faría and Ackerman 1999).

Mechanisms of self-pollination

Although orchids are widely considered to exemplify sophisticated floral adaptations for outcrossing, species capable of autogamy (automatic self-pollination) occur in virtually every tribe and subtribe (Catling 1990). Autogamy in orchids is facilitated by the close proximity of anther and stigma and many of the mechanisms of autogamy involve modification of the pollinarium. In orchids with sessile pollinia, the massulae are often friable and fall onto the stigmatic surface, sometimes even in the bud stage (Kurzweil and Johnson 1993). In some cases whole pollinia fall or slide onto the stigmatic surface (Mehrhoff 1983). Caudicles in autogamous species are often either weak, allowing pollinia to flip across onto the stigma when the flower is jarred, or undergo a bending movement which brings the pollinium into contact with the stigma of the same flower. A similar effect is achieved by bending of the stipe in, for example, *Oncidium*

glossomystax Reichb.f. (van der Pijl and Dodson 1966).

There are differing degrees of autogamy among orchids. Most retain the potential for outcrossing by having functional pollinaria that will self-pollinate the stigma only if it is not removed by insects. Only a few orchids are truly cleistogamous or apomictic (Catling 1990). Autogamy is most frequent among orchids with a weedy habit or those that occur in habitats that are marginal for pollinator activity (Hagerup 1952, Johnson et al. 1994, Williamson 1984). Catling and Catling (1991) showed that autogamy in North American orchids is more common at higher latitudes.

Pollinaria: a key innovation in the radiation of orchids?

In terms of numbers of species, the *Orchidaceae* are rivalled only by the *Asteraceae*. The *Orchidaceae* today numbers between 18 000 and 25 000 species (Atwood 1986). The most recent estimate is that of Dressler (1993) who gives 19 500 species for the family.

Pollinia are not unique to the *Orchidaceae*. An analogous structure has evolved in the *Asclepiadaceae*, and it is instructive to ask whether these families share common evolutionary outcomes as a result of their independent evolution of pollinia. The answer is a disappointing "no". The flowers of *Asclepiadaceae* are invariably radially symmetrical, as opposed to the bilateral symmetry of orchids. Certainly asclepiads have not shown the same rampant speciation that has been evident in the orchid family, although the richness of *Asclepiadaceae* exceeds that of orchids in semi-arid regions. Perhaps the comparison is meaningless, since the two families differ in so many other intrinsic characteristics, such as growth form and floral symmetry. It is nevertheless interesting that many *Asclepiadaceae* are quite promiscuous in their pollination systems, suggesting that the association between pollinia and specialized pollination systems is not an obligate one.

The evolution of pollinaria

Although monads and pollinia represent the two extremes on either end of the pollen clumping scale, there are many intermediate conditions among the angiosperms. Pollen occurs in tetrads in several families and dispersal of pollen in clumps is achieved by viscin threads in Onagraceae and Ericaceae, filamentous threads in Strelitziaceae and polyads in mimosoid legumes. One of the implications of pollen clumping is that the chances of multiple paternity are reduced. Studies of orchids with solid-type pollinia show that the vast majority of flowers are pollinated by just one pollinium (Nilsson et al. 1992b, Andersson 1999). Kress (1981) argued that single paternity may confer advantages to plants with pollen dispersed in clumps because competition is reduced among seeds which are full siblings. He pointed out that the trend for wind-pollinated plants to be uni-ovulate may be a consequence of selection to avoid competition among seeds. The converse argument is that multiple paternity encourages gametophytic competition among pollen grains for access to ovules and therefore ensures fitter offspring, particularly when resources are limiting and ovule abortion is a necessity (Ellstrand 1984).

Genetic implications aside, it is clear that pollen dispersal in clumps is associated with greater ovule numbers in families such as Orchidaceae, Asclepiadaceae, Onagraceae and Ericaceae (Cruden 1977, Kress 1981). There are two ways of interpreting this trend: either selection has favoured many small seeds, in which case pollen clumping is a secondary adaptation to ensure fertilization of large numbers of ovules or, alternatively, selection has favoured pollen clumping and the evolution of large number of ovules is a secondary adaptation to ensure fertilization by large numbers of pollen grains. We will explore the merits of both of these hypotheses.

1. The pollen export hypothesis. Successful export of pollen to conspecific stigmas is important for the male component of reproductive success, and selection should thus

favour any modifications that enhance pollen export.

Studies of species with loose pollen have revealed substantial losses through pollen falling off the bodies of insects, or deposited on foreign stigmas. There is now ample evidence that a very high percentage (typically more than 99%) of the pollen of xenogamous angiosperms is wasted during transport. For example, Rademaker et al. (1997) found that only 0.15% of pollen of *Echium vulgare* was deposited onto stigmas, less than 0.5% of pollen removed from the flowers of *Rhexia virginica* L. (Melastomataceae) was deposited on stigmas (Larson and Barret 1999) and only 0.52% of the pollen removed from flowers of *Erythronium grandiflorum* Benth. was deposited on stigmas (Thomson and Thomson 1989). Such wastage could lead to the evolution of higher pollen:ovule ratios or mechanisms such as pollen clumping that minimize pollen wastage. Estimates of the probability of pollen reaching a stigma range from 20% in *Cymbidiea flabellata* Rolfe. (Nilsson et al. 1986) to 51% in some populations of *Cypripedium acuale* Ait. (O'Connell and Johnston 1998).

The efficiency of pollen export in orchids with solid pollinaria that are deposited in their entirety can be estimated by the ratio of pollinaria removed and deposited. Estimates of the efficiency of pollinia export in orchids with sectile pollinaria are made more difficult by the fact that a single pollinarium can pollinate several flowers.

Pollinaria may also promote pollen export when pollinator visits are infrequent, as the entire pollen complement of a flower can be removed in a single visit. Studies of plants with loose pollen show that only 7–65% of pollen in an anther is removed in a typical visit, thus leading to low removal efficiency when a flower is visited just once (de Jong et al. 1992). Indeed, it has been argued that attraction of repeated pollinator visits to ensure efficient pollen export has been an important factor in the evolution of floral traits in plants with loose pollen (Bell 1985). Data for orchids show that pollinator visits are often infrequent

(Johnson and Bond 1997, Neiland and Wilcock 1998), perhaps as a consequence of low population densities and non-rewarding pollination strategies, and this may have promoted the cohesion of pollen into pollinia to ensure efficient pollen removal. Conversely, when pollinator visits are frequent it may pay to dispense pollen in small amounts and thereby spread the risk among several vectors (Thomson et al. 1989).

Although, pollen clumped into pollinia is unlikely to end up on foreign stigmas, especially of non-orchids, there is always the risk that a generalist vector will not visit another flower of the same species and therefore squander all of the pollen from a flower. Ensuring that the pollinium reaches its target destination is obviously important to male fitness of orchid flowers and may have driven selection for the specialized pollination systems that are characteristic of the Orchidaceae. The trend noted by Tremblay (1991) for pollination systems to be more specialized in orchids with solid pollinaria than in orchids with mealy pollen, such as the Cypripedioideae, is consistent with this idea.

One of the ways in which pollen is lost from typical angiosperm flowers is through the activities of pollen-collecting insects. Pollen packaged in pollinia is generally unavailable to pollen-collecting insects, although recent studies show that bees may actively collect the pollen of some orchids with loose pollen masses, such as *Cleistes divaricata* (L.) Ames and *C. bifaria* (Fernald) Catling and Gregg (Gregg 1991). Other orchids, such as some *Maxillaria*, *Polystachya* and *Cephalanthera* species, attract pollen-collecting insects by falsely advertising the presence of pollen through modifications of the labellum (van der Pijl and Dodson 1966, Dafni and Ivri 1981).

In their hypothetical sequence of the evolution of orchids in which great importance is placed on pollinator driving forces, van der Pijl and Dodson (1966) consider small seeds to have evolved subsequent to pollinaria. They cite the Asclepiadaceae as an example of a

family in which pollinia have evolved despite having large non-mycotrophic seeds.

In summary, the pollen export hypothesis is supported by data which show that pollinaria can improve male reproductive success through efficient removal from the anther, minimal wastage due to pollen falling or being groomed off the bodies of pollinators, and a high probability of being deposited on a conspecific stigma.

2. The small seeds hypothesis. A pivotal aspect in the evolution of the Orchidaceae has been the reduction of the seeds and their contents to an absolute minimum. In all but a few species, endosperm and a recognizable embryo are absent from the seed. This loss of reserves must have occurred in conjunction with increasing reliance on fungal symbionts. The reduced energy investment per ovule meant that more ovules could be produced. For such a system to be successful, fertilization capacity must be enhanced through the deposition of much larger amounts of pollen on the stigma, as can be achieved through the evolution of pollinaria (cf. Neiland and Wilcock 1995, Nazarov and Gerlach 1997).

Dressler (1981) argued that small seeds (associated with the mycotrophic habit) would have preceded (and provided a powerful stimulus for) the evolution of pollen clumping that is ultimately expressed in pollinia. Similar views were expressed by Darwin (1877) who considered pollinia to have evolved subsequent to small seeds in orchids. Recent evidence indicates that the small-seeded Apostasioideae are indeed the sister group to the remainder of the Orchidaceae (Cameron et al. 1999), and there are thus good phylogenetic grounds for believing that small mycotrophic seeds were the initial evolutionary impetus for the evolution of pollinaria.

The evolution of the epiphytic habit in orchids would have required very light dustlike seeds to effectively colonize new growth sites in forest canopies (Dressler 1981). Numerous small seeds are also found in other largely epiphytic families, such as the Bromeliaceae and Gesneriaceae. While pollen is not clumped

in these families, many of the species are pollinated by vertebrates which would be expected to deposit large enough stigmatic pollen loads to ensure fertilization of the large number of ovules.

The reduction of fertile anthers from six to one, and the close alliance of male and female whorls in families such as Cannaceae, Marantaceae, Costaceae and Zingiberaceae is remarkably similar to Orchidaceae. Within these lineages there is an overwhelming reduction of tepals and their replacement by petaloid staminodes which are frequently modified into a labellum remarkably similar to those of Orchidaceae. Yet the pollen of these families does not cohere into pollinia. The key functional difference may be the mycotrophic habit of Orchidaceae which is pivotal in the evolution of dust seed in this slow growing group.

Conclusions

The most likely evolutionary scenario for orchids is that both small seeds and the need for efficient pollen export were complementary selective pressures for the evolution of pollinaria. Ancestral orchids probably had small seeds which would have promoted the cohesion of pollen as it leads to greater stigmatic pollen loads. At the same time, infrequent visitation by insects would have favoured a pollen packaging strategy that allowed removal of all of the pollen from the anther in one visit with minimal wastage in transit and minimal probability of it being deposited on foreign stigmas.

The innovation of pollinaria undoubtedly led to greater floral specialization in the orchids (Tremblay 1991), and thereby contributed indirectly to pollinator-driven speciation in the family (cf. Johnson et al. 1998a). In addition, the phenomenally high number of recombinants possible after each successful pollination event in orchids would have supplied the variation on which natural selection can act.

On the other hand, pollinaria impose certain constraints on the evolutionary options within the family. Pollination by pollen-collecting bees is rare among orchids, with the

exception of a few species with powdery pollen, such as *Apostasia* (Dressler 1986) or loose pollen masses such as *Cleistes* (Gregg 1991). Likewise, wind-pollination is not viable for orchids due to the aggregation of pollen into weighty units. The ability of orchids to utilize promiscuous pollinators is limited because of the risk of losing the entire pollen complement of a flower to an unreliable vector. Orchids have followed an evolutionary pathway of specialized pollen transfer and have flourished in terms of numbers of species, but at the same time have failed to dominate ecosystems in the way that wind-pollinated plants, such as the Poaceae, or those with generalist pollination systems, such as the Asteraceae, have been able to do.

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Deceptive orchids with Meliponini as pollinators

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Abstract. Visitation of orchids by Meliponini (stingless bees) is confirmed only in 13 *Melipona*, *Partamona* and *Trigona*, for *Xylobium* and *Maxillaria*, with the addition of *Trigona fulviventris* visiting *Ionopsis*. Some bees evinced multiple floral visitation by carrying several stipes and viscidia from pollinaria, thus may cause seed set. None foraged pseudopollen, nor is collection of this substance by bees verified. Meliponine-visited orchids had pollinia in quartets with emplacement on the bee's scutellum, possibly devices for pollinia survival on a social bee passing through its nest. Further, orchids produced no nectar, but bees repeatedly came to flowers. A testable basis for the orchid-meliponine relationship is mimicry of rewarding resources, or bee pheromone mimicry, recently documented for some honey bees. Meliponine pheromone analogs (nerol and 2-heptanol) are here noted for *Maxillaria*, but lack of foraging with pheromones by *Melipona* suggests multiple avenues of mimicry by orchids, including alarm pheromone and carrion mimicry.

Key words: Neotropical orchids, Meliponini, mimicry, pollination.

Pollination of orchids by bees was summarized by Pijl and Dodson (1966), who closed with the prediction that 8% of orchids would be found pollinated by social bees. Particularly, more data on visitors in the genera *Apis* and *Bombus* were anticipated. Although reports mention *Apis* visiting orchids (see e.g. Sasaki

1992, Roubik 1996), by far the largest share of the literature has been devoted to formidably diversified pollinarium placement on, and the attraction of, male Euglossini in the neotropics (Pijl and Dodson 1966; Dressler 1982, 1993; Ackerman 1983; Williams and Whitten 1983; Roubik 1989; Eltz et al. 1999). The euglossines *Eulaema* and *Euglossa* have many social species, but they lack the large and permanent colonies of some tropical *Bombus*, *Apis* and Meliponini (Roubik 1989). The biology of interaction with orchids must differ among these groups. In this paper I present new records of orchid visitation by neotropical stingless bees which are not gathering pollen or nectar from orchids. Perhaps surprisingly, all are in the Isthmian and Andean region, including the rim of the western Amazon, while none are from the lowlands of the greater Amazon basin, where most Meliponini are found. The emerging orchid-Meliponini "syndrome" appears to range from Bolivia to Central America. We still know little of how this interaction operates or its significance to orchids, but at this stage it is useful to integrate new data with old, and to codify impressions.

Much of the present information is limited to bee species and flower genera (see Roubik and Moreno 1991) because my observations were of pollinaria on foraging bees either

visiting non-orchid flowers or entering their nest. Such data complement findings of Dodson (1962, 1965) and Dodson and Frymire (1961a, b) who observed bees at orchid flowers of known species. Although unable to name the species of orchids involved, I do discuss the specific meliponine taxa, along with features that define this unusual ecological interaction. Its mode of operation apparently involves deception on the part of orchids, with no exchange of resources such as 'pseudopollen' – erroneously suggested by the literature.

The orchids and stingless bees

Orchids confirmed having repeated association with stingless bees are almost exclusively epiphytic (Dressler 1993) and belong to the tribe Maxillarieae (Epindendroideae). The taxon is advanced, as are orchids visited by euglossine bees (a separate clade within the corbiculate bees, to which Meliponini, Bombini and Apini also belong – Koulianatos et al. 1999, Dressler 1993). Within Maxillarieae are two genera in subtribes Lycastinae (*Xylobium*) and Maxillariinae (*Maxillaria*) associated with three genera of worker Meliponini (*Melipona*, *Trigona* and *Partamona*). There are three to five species recorded with orchid pollinaria in each bee genus (Table 1).

Neither *Xylobium* (for an exception see Pinatudi et al. 1990) nor *Maxillaria* (*M. coccinea* is an exception, J. Ackerman, pers. comm.) produce nectar, both have four flattened pollinia with a viscidium (Pijl and Dodson 1966, Dressler 1993), and the former but only some of the latter have a prominent stipe (Rodríguez et al. 1986). All species I have examined (apparently three) have prominent stipes (Fig. 2A, C). In contrast, the stipe of *Xylobium* is broad but barely attains the length of the hairs fringing the bee's scutellum where it attaches (Fig. 2B, D). One other genus, identified here as *Ionopsis* (Table 1), also is nectarless (Ackerman 1986) but has two small, globose pollinia on its outer surface (when on the bee's thorax) near the apex of a long, broad stipe.

Pollinarium placement

The pollinarium is centered on the scutellum of the bee (Figs. 1–3). As the bee backs out of the flower (Dodson and Frymire 1961a, Pijl and Dodson 1966), the rostellar flap is contacted and a stigmatic liquid cements the viscidium of the pollinarium to the bee's thorax. Entering the next flower, the stigmatic cleft, placed farther within the flower than the rostellar flap, collects pollinia carried on the bee's dorsum. If the bee visits several orchid flowers in succession, stipes and viscidia accumulate, superposed, on the scutellum (Table 1 and Fig. 3). This is good evidence of pollination but not of outcrossing, as multiple flowers, along with relatively site-constant group-foraging bees (see Roubik 1989) are present on an individual plant – e.g. *Trigona amalthea* on *Maxillaria latilabrum* (Pijl and Dodson 1966). Two to four viscidia and stipes, with all or some pollinia removed, were upon bees listed in Table 1. I cannot verify whether pollinia were removed from these pollinaria by bees within the bee nest, or by orchid stigma.

Scutellar placement of pollinaria is uniform among stingless bees, and also on honey bees (pers. obs.) In marked contrast, the largest genus of orchid bees *Euglossa* (ca. 100 spp.) with most the same size as *Apis* or *Melipona* tend not to carry pollinaria on the scutellum, but at many other locations upon body, legs and antennae (Ackerman 1983, Dressler 1993). There is some overlap in pollinarium sites on bees of these groups, with scutellar emplacement on euglossines by species of *Acineta*, *Stanhopea*, *Vanilla*, *Gongora* and *Sobralia*. Pijl and Dodson (1966: 41) give only one example of an orchid (*Bletia*) visited both by *Melipona* and *Euglossa*. Attraction of euglossines and meliponines is presumably due to different sets of chemicals or other cues, and emplacement on the scutellum is evidently not driven primarily by the size of the bee. Moreover, a few of the orchid species mentioned by Pijl and Dodson (1967) and at least one that I have repeatedly encountered in Panama, are visited both by *Trigona* and *Partamona*. As discussed

Table 1. New and previously recorded associations between stingless bees (Meliponini) and neotropical orchids

Visitor & presumed pollinator*	Orchid host	Locality and reference**
<i>Melipona eburnea</i>	<i>Maxillaria</i>	Ecuador: Dodson 1962
<i>Melipona fuliginosa</i>	<i>Cattleya</i>	Peru: Dodson 1965
<i>Melipona fuliginosa</i>	<i>Maxillaria</i>	Colombia: Leticia 200 m
<i>Melipona fuliginosa*</i>	<i>Maxillaria</i>	Costa Rica: Cerro Nara
<i>Melipona fuliginosa*</i>	<i>Maxillaria</i>	Panama: Carti Rd. 200 m
<i>Melipona panamica</i>	<i>Maxillaria</i>	Panama: Fortuna 1100 m
<i>Melipona panamica*</i>	<i>Maxillaria</i>	Costa Rica: Volcan Cacao 1200 m
<i>Partamona aequatoriana*</i>	<i>Maxillaria "A"</i>	Ecuador: Pichincha 2500 m
<i>Partamona musarum</i>	<i>Xylobium</i>	Panama: Cerro Campana 700 m (May, Oct, Dec)
<i>Partamona nigrior</i>	<i>Schomburgkia</i>	Peru: Dodson 1965
<i>Partamona orizabaensis</i>	<i>Xylobium</i>	Panama: Fortuna 1100 m
<i>Partamona testacea</i>	<i>Maxillaria "B"</i>	Peru: Dodson 1965
<i>Plebeia minima</i>	<i>Sobralia</i>	Brazil: Ducke 1902
<i>Plebeia droryana</i>	<i>Trigonidium</i>	Brazil: Kerr and Lopez 1963
<i>Scaptotrigona postica</i>	<i>Xylobium</i>	Brazil: Pintaudi et al. 1990
<i>Trigona amalthea</i>	<i>Maxillaria</i>	Ecuador: Napo, Rio Hollin 700 m
<i>Trigona amalthea</i>	<i>Xylobium</i>	Costa Rica: Dodson 1965
<i>Trigona amalthea</i>	<i>Xylobium</i>	Costa Rica: Pitilla, Volcan Cacao 700 m
<i>Trigona amalthea</i>	<i>Xylobium</i>	Peru: Dodson 1965
<i>Trigona amalthea</i>	<i>Xylobium</i>	Venezuela: Bolivar, Sta. Elena 600 m
<i>Trigona corvina*</i>	<i>Maxillaria</i>	Panama: Sta. Rita Ridge 500 m (May, Sept)
<i>Trigona corvina</i>	<i>Maxillaria</i>	Panama: Cerro Campana 700 m (May, Oct, Dec)
<i>Trigona ferricauda</i>	<i>Maxillaria "A"</i>	Ecuador: Pichincha 2500 m
<i>Trigona fulviventris</i>	<i>Ionopsis</i>	Panama: Pipeline Rd. 100 m
<i>Trigona fulviventris*</i>	<i>Ionopsis</i>	Costa Rica: Volcan Cacao 1200 m
<i>Trigona fulviventris*</i>	<i>Ionopsis</i>	Panama: Sta. Rita Ridge 500 m
<i>Trigona silvestriana</i>	<i>Maxillaria "B"</i>	Peru: Dodson 1965
<i>Trigona silvestriana</i>	<i>Xylobium</i>	Bolivia: Cochabamba 200 m
<i>Trigona silvestriana</i>	<i>Xylobium</i>	Peru: Tingo Maria 300 m

* Multiple stipes, superposed, adhering to the scutellum, with pollinia partly or completely removed

** Elevations given for new records; collections made by author except for those noted in Costa Rica (InBIO collection, Heredia, Costa Rica), and Pichincha, Ecuador (National Pontifical Catholic University collection, Quito, Ecuador)

below, while not greatly different in size, the fact that two genera visit the same flowers bears strongly on any explanation for stingless bee visits at these orchids.

The structure and placement of pollinaria on meliponines suggest orchid adaptations peculiar to social bees living in large colonies. Two points are central. First of all, the viscidium wraps completely around the outer rim of

the scutellum and reaches the adjacent axillae (Fig. 2B–E). Stout fringing hairs on the scutellum often conform to the length of the viscidium, which cups these structures. While the stipe may project beyond the scutellar hairs of some *Maxillaria* (Fig. 2A, C), in both *Maxillaria* and *Xylobium* it can also correspond perfectly to their length. Secondly, the pollinia tend to be flat, and clustered with the inner pair

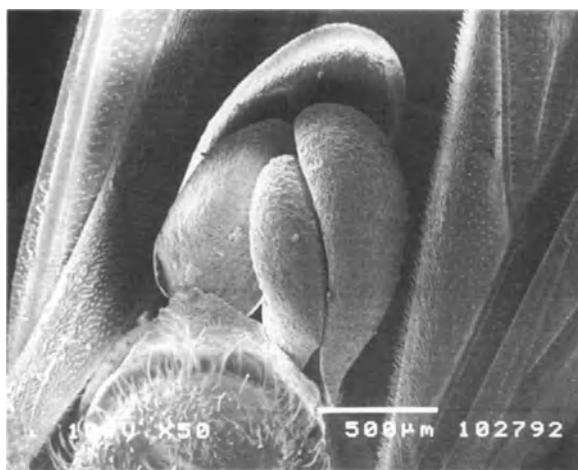


Fig. 1. Scutellum and wings of *Trigona corvina*, showing pollinarium stipe and four pollinia of *Maxillaria* carried on the scutellum

smaller and the outer pair concave on inner surfaces, sheltering the smaller pollinia. Pollinia of *Maxillaria* on stingless bees often differ greatly in size within the pollinarium, while those of *Xylobium* tend to be subequal in length and similar in width. However, *Xylobium* sometimes has the inner two pollinia concealed beneath the outer pair, or closely appressed to these, forming a compact bundle.

Orchid reproduction via highly social stingless bees or honey bees may result in selective advantages for pollen placed in four pollinia, with outermost pollinia concealing or partly protecting the inner pair. This number of pollinia is plesiomorphic for orchids and intermediate among neotropical orchids (Dressler 1993, Rodriguez et al. 1986). Pollinia carried in quartets may better survive the trip on the scutellum of a social bee through its nest, where any foreign body is promptly removed by nest mates. The tiny pollinia of *Ionopsis*, although only two in number, are well protected and partly hidden by the cowl-like stipe that holds them. Placing a pollinarium strictly on the ventral lip of the scutellum seems the best means available for avoiding pollinarium removal by individual foraging bees. This part of the body is not reached during self grooming. In addition, the form and placement of pollinia likely hide them

from the worker bee's nest mates, or make it difficult to remove all pollinia in one bite. Stingless bees continue to forage throughout the day, making several return trips to the nest, thus the chances of pollen transfer are increased for orchids able to place their pollinia on relatively safe sites.

Bee Attraction and Deception

Maxillaria – over 50 species in Ecuador alone of the 300 neotropical taxa (Pijl and Dodson 1966) – often has pseudopollen on the lip of the floral corolla. Dodson and Frymire, and also Dodson, cited by Pijl and Dodson (1966), and Dressler (1993: 27), state that bees gather such pseudopollen. However, none of the literature contains reference to the bees involved or the behavior witnessed. I could find no published information that clearly substantiates the attraction of pseudopollen or its collection by any bee. Nonetheless, Pijl and Dodson (1966: 202) define pseudopollen as a deceptive structure that mimics pollen or anthers, ostensibly to induce visitation by insects. They state elsewhere (1966: 24, 76) that *Maxillaria* provides starch cells collected by bees. Dressler (1993) refers to starchy cells collected by bees from *Polystachya* (Malaxidae or Polystachyeae, depending on systematic interpretation). This genus also has four pollinia, a viscidium and a short but distinct stipe (Rodriguez et al. 1986). From my observations, these suggestions are not corroborated, because bees collected with pollinaria of *Maxillaria* and other orchids carried no pseudopollen (or non-pollen of any kind) in the corbicula of the hind leg, where pollen destined for brood provisions is transported. Only five of the 24 specimens of stingless bees included in Table 1 had what appeared to be pseudopollen on the thorax or abdomen (Fig. 2A). The non-pollen substance that I observed was approximately 40 microns in greatest dimension and had an irregular, oblong shape (Fig. 2A). However, I did not compare it to pseudopollen collected from a flower of *Maxillaria*, and it was not placed on the venter of

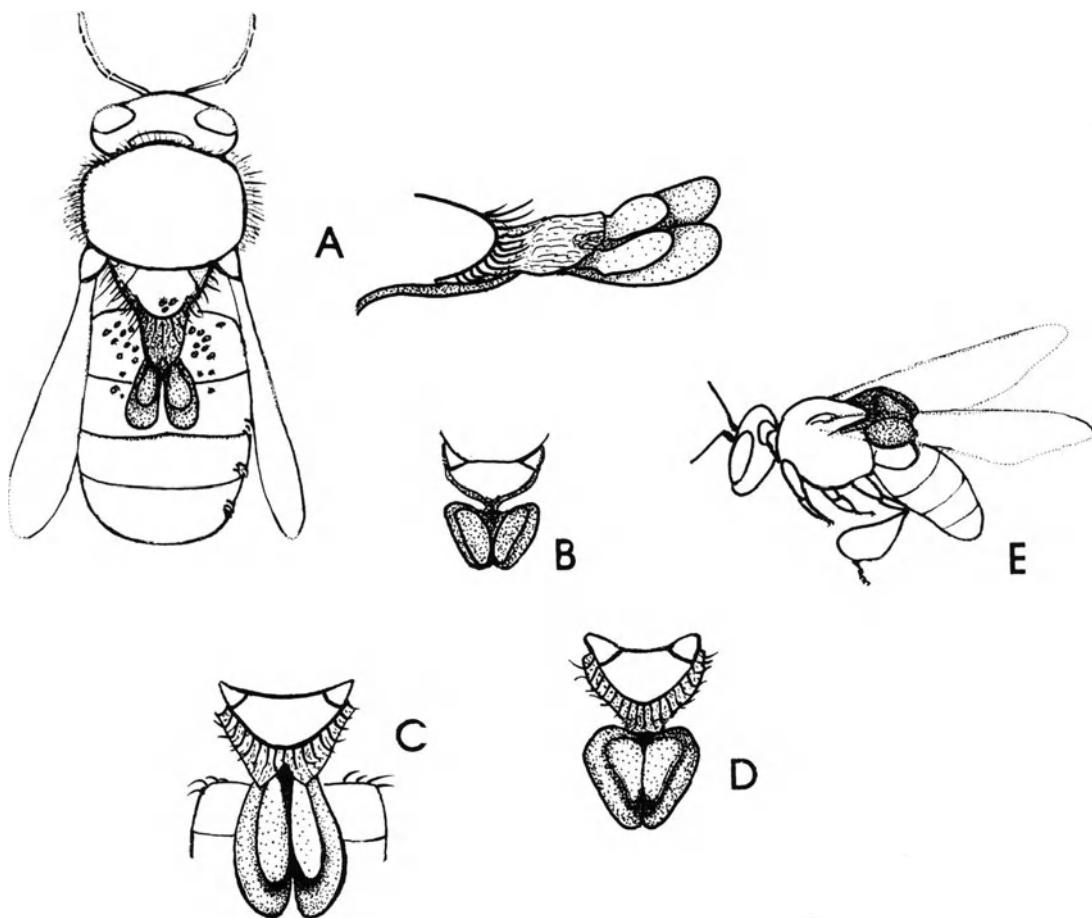


Fig. 2. Stingless bees, presumptive pseudopollen and pollinaria placement: **A** *Melipona fuliginosa* with pollinarium of *Maxillaria* on the scutellum and pseudopollen scattered on the metasoma and scutellum; **B** scutellum of *Partamona* with the pollinarium of *Xylobium*; **C** scutellum of *Melipona panamica* with the pollinarium of *Maxillaria*; **D** scutellum of *Trigona silvestriana* with pollinarium of *Xylobium*; **E** *Partamona musarum* with pollinarium of *Xylobium*

the bee, where it would normally be deposited if a bee entered the corolla in the usual way. There is some chance that the substance I believe is orchid pseudopollen is another material (although this seems unlikely). Perhaps no stingless bee transports pseudopollen for the nutritional needs of the colony. Whether other bees do so is an open question. Three of the bees examined, *M. fuliginosa*, *T. amalthea* and *T. corvina*, had genuine pollen in their corbicula, while also carrying orchid pollinia. This situation is consistent with deception involving orchid mimicry (see following).

Orchid pollination by bees involves different mechanisms, both physical and chemical,

but there seems no reason that the attractants, mechanisms, and species involved cannot be numerous or redundant. Roughly 700 of the several thousand neotropical orchids participate in the androeuglossophilous (euglossine bee) pollination syndrome (Ackerman 1983), while euglossines are limited to about 200 species (Roubik 1989, Dressler and Ospina Torres 1997, Ospina Torres and Sandino-Franco 1997). In equatorial forests, the honey bees, stingless bees and other apids, as well as megachilid bees (personal observation in Gabon), fly to orchids and carry their pollinaria (Pijl and Dodson 1966, Roubik 1996). The possibilities of nectar production and floral

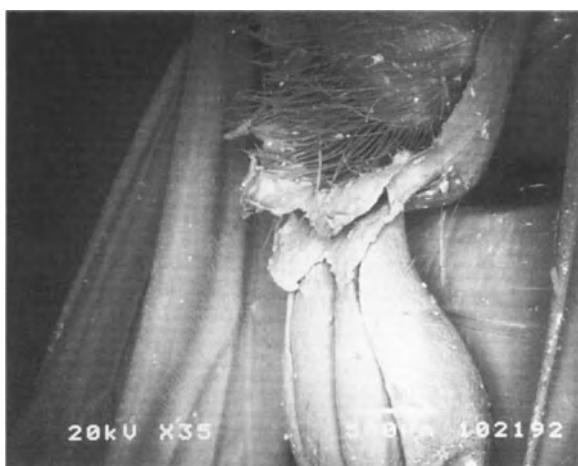


Fig. 3. Two stipes on the scutellum of *Trigona corvina* carrying pollinia of *Maxillaria*

mimicry exist in virtually all the less well-documented interactions. While male orchid bees apparently use orchid chemicals to convince potential mates that they have relatively high fitness and 'good genes' (Eltz et al. 1999, Roubik 1999), study of orchids and *Apis cerana* in Japan has given evidence that orchids attract and deceive social bees with 'aggregation pheromone' mimics, containing the same components that are products of the honey bee Nasanov gland (Sasaki 1992, Sakagawa and Mutsuyama 1999). *Apis* workers, drones and even reproductive swarms visit flowers of *Cymbidium* that produce such chemicals. *Trigona* use pheromones extensively when recruiting nestmates to resources, and *Partamona* also is believed to do so, but *Melipona* does not generally utilize odors to guide nestmates (Roubik 1989, Nieh and Roubik 1998). Thus, while mimicry of odor trails or pheromones attracting foraging nestmates may appear in the fragrances of orchids attractive to meliponine visitors, the subject may hold further complexity. Multiple bee genera visiting the same orchid species signifies that, while Meliponini have rather distinctive foraging odors at the level of species or even genus, some chemicals (or visual characteristics of flowers) attract diverse species. For the evolution of a sustained interaction with orchids, a more fundamental question involves bee size and

pollinarian placement, as a prelude to sophisticated chemical mimicry or attraction of only a limited set of bees by orchids. *Trigona*, *Partamona* and *Melipona* are medium to large in size among stingless bees, and have a scutellum that projects backwards, making a convenient shelf on which to paste the orchid viscidium. The honey bee pollinators of orchids also carry pollinaria uniquely on the scutellum. As I argue above, the scutellum may offer a safe site.

Stingless bees are likely deceived by orchids that offer neither carbohydrate nor protein. Close visual mimicry of rewarding flowers, a tactic used by *Oncidium*, *Odontoglossum* and *Ornithocephalus* that mimic the oil flowers of certain neotropical Malpighiaceae sought by centridine bees and *Paratetrapedia* (Apidae), is a well-known phenomenon (Pijl and Dodson 1966: 41, Roubik 1989). However, I suggest that meliponine interest in orchid flowers is likely caused by odors mimicking bee exocrine gland chemicals, including pheromones used in foraging or nest defense. This untested idea is given some support by the correspondence between odors produced by *Maxillaria* flowers sampled by Kaiser (1993, see Table 2) and the alarm or foraging pheromones known from *Trigona* and *Melipona* (Roubik 1989, see Table 2). The chemicals listed here include nerol and 2-heptanol, although not necessarily from a flower species visited by Meliponini. The data are at least suggestive. Little work has been done on the pheromones of *Partamona*, thus it is not possible to compare its glandular products to orchids.

Odor deceit causes flies to visit some orchids (Pijl and Dodson 1967: 31). They are attracted by a stench resembling carrion. Many stingless bees visit carrion; some are relatively specialized in this trait (Roubik 1989). It does not seem coincidental that *Melipona*, *Partamona* and *Trigona*, all of which forage upon dead animals, also are attracted to certain orchid flowers. Why only *Partamona*, *Melipona* and *Trigona* (from about 20 genera constituting neotropical Meliponini) often associate with orchids offering no reward

Table 2. Chemical composition of the floral fragrances produced by *Maxillaria variabilis* ('dark red' and 'yellow' forms, Kaiser 1993), possibly visited or pollinated by Meliponini. Correspondence to known components of worker stingless bee mandibular gland odors is indicated with an “**”, and the bee genus (Roubik 1989)

Compound	GC/MS relative intensities (%)
α -pinene	minute; 1.2
hexanal	minute
β -pinene	minute; 1.6
sabinene	1.0; minute
myrcene	1.4; minute
heptan-2-one	4.0
(Z)-3-hexenol	minute
(Z)-4-decenal	minute
(E)- β -farnesene	5.3
limonene	3.0; minute
citronellal	3.1
eucalyptol	1.0
(E)-ocimene	1.0; 3.0
octanal	minute
2-heptanol * <i>Melipona</i> , <i>Trigona</i> , <i>Scaptotrigona</i>	3.9
6-methyl-5-hepten-2-one	4.1; minute
nonanal	2.3; minute
neral* <i>Geotrigona</i> , <i>Lestrimelitta</i> , <i>Trigonisca</i>	22.5
geranial* <i>Geotrigona</i> , <i>Lestrimelitta</i> , <i>Trigonisca</i>	21.7
α -copaene	10.9; 2.0
decanal	3.8; minute
benzaldehyde* <i>Scaptotrigona</i>	minute
linalool	minute
caryophyllene	minute
germacrene D	39.5; 39.5
bicyclogermacrene	1.9; minute
benzyl acetate	minute
methyl salicylate	minute
(E)-geranylacetone	3.9
benzyl alcohol	minute
phenylethyl alcohol	minute
(E)-nerolidol	minute
nerol* <i>Trigona</i> , <i>Melipona</i>	1.2
geraniol	1.1
γ -decalactone	minute; minute
benzyl benzoate	minute

is by no means resolved. Due to their high abundance and speciosity (30–50 taxa), these genera may seem important to orchids primarily as a sampling artifact. Many species of *Plebeia*, *Lestrimelitta*, *Scaptotrigona*, *Trigonisca*, *Paratrigona*, *Nannotrigona*, *Oxytrigona*

and proximate clades of *Trigona* (*Tetragona*, *Geotrigona*, *Ptilotrigona*, *Cephalotrigona*, *Friesemelitta*) may associate with orchids, yet remain obscure. I have seen one example of the last three genera with an unidentified pollinarium, apparently not an asclepiad.

Scaptotrigona and *Plebeia* do associate with orchids in the Amazon basin (Table 1).

With about 10 species and as many published reports of orchid visitation, the genus *Apis* seems unlikely to contribute substantially to orchid reproduction in the tropics or subtropics. The potential for orchid pollination among 450 species with over 800 distinctive geographic populations of Meliponini is far greater. My observations at the nests of at least *Melipona* and *Trigona* made me realize that stingless bees are not merely "bumping into" a few orchids and visiting them. A single nest displayed quite clearly the interaction between Meliponini and Orchidaceae. A few bees each minute, over a period of a few hours, entered the nest with pollinaria on their scutellum. With few literature citations for their visitation of orchids, and all those of Brazil involving nectar use (Ducke 1902, Kerr and Lopez 1963, Pintaudi et al. 1990) Meliponini and non-rewarding orchids seem a neglected research topic. Such basic questions as whether the orchids in question are fragrant, are visual mimics of other flowers in the habitat, offer mealy non-pollen substances collected or eaten by bees at the flowers themselves, or produce fruit and seed resulting from bee transfer of pollinaria, need further field study and clarification.

It is remarkable that no bees utilize pollen grains grouped in pollinia, and that many of the same flowers do not produce nectar; this accentuates the importance of non-food attractants to pollinators of orchids. The geographical range of Meliponini that visit orchid flowers seems limited to primarily extra-Amazonian areas, but this perception may change. In the neotropics alone there may be numerous or at least widespread orchid species pollinated by Meliponini, with a potential for chemical and morphological adaptation by orchids as complex as that recently discovered for *Apis cerana* in Asia. As already mentioned, such chemicals might resemble alarm attractants or other pheromones (Table 2), like the nerol and 2-heptanol made by *Maxillaria*, *Melipona* and *Trigona*. The topic is ripe for

research. Along with chemical and other styles of mimicry, the form, pollinia number and placement of pollinaria suggest response of orchids to the social lives of their pollinators.

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Thread-forming structures in angiosperm anthers: their diverse role in pollination ecology

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Abstract. This paper reviews the origin, nature, systematic distribution, and the respective function of the highly variable and diverse thread-forming structures in angiosperm anthers (including somewhat similar, rare features in ferns and gymnosperms). On one hand, such threads may function as pollen-connecting vectors in forming pollen dispersal units, as sporopollenin threads (viscin threads), e.g. in Onagraceae, or sporopollenin-less threads in surprisingly many other angiosperm families. On the other hand, as is known from the *Impatiens* – “pollen basket”, threads or ropes may be involved in pollen presentation. In addition, for the first time two new examples of “pollen baskets” in Boraginaceae and Scrophulariaceae are reported. In *Echium* the basket is formed by cellular elements from the modified septal regions, whereas in *Esterhazya* a similar effect is achieved in an analogous manner by trichomes of the epidermal layer of the thecal wall. There is obviously a different function of these seemingly very similar baskets: in *Echium* the feature acts preferably as a pollen presentation agent, whereas in *Esterhazya* the primary function is to prevent all the pollen from being dispersed too soon.

Key words: Angiosperms, *Echium*, *Esterhazya*, pollination ecology, anthers, pollen, secondary pollen presentation, sporopollenin, viscin threads.

In zoophilous taxa, the simultaneous transfer of larger pollen associations certainly increases

the probability that ovules will be fertilized (Knox and McConchie 1986, Pacini and Franchi 1998, Pacini and Franchi 1999, all for review). Pacini and Franchi (1996) have listed types of pollen dispersing units in relation to important aspects of pollination ecology. Mature pollen of anemophilous taxa is dispersed simply as individual pollen grains without any additional feature or auxiliary means. In contrast, in zoophilous taxa there are many special anatomical features of anther and pollen morphology to sustain or to hinder the effective pollen transport, e.g. various forms of pollen dispersal units, or special features in pollen presentation. These features directly relate to pollination ecology, such as the widely occurring secondary pollen presentation (Yeo 1993). The present paper focuses on the different aspects of anther-borne features that appear in a thread-like manner.

Anther-deriving threads or fibers are manifold in origin and do not only connect individual pollen grains. Anther-deriving fibers not related to pollen-connecting threads may play another significant role in pollination ecology, either to hinder pollen grains from being dispersed in an untimely way from the opened pollen sac, or to act as a pollen presenting feature. There may be other functions, too, or even no detectable function at all

(*Gymnocalycium*, Halbritter et al. 1997). The evolution, form, and function of pollen-related anther-borne threads will be reviewed in the present paper; two novel, remarkable cases are added (*Echium*, *Esterhazya*).

Material and methods

The plant material of the *Echium* species (*E. auberianum* Webb et Berth., *E. russicum* J. F. Gmelin, *E. cf. virescens* DC., *E. vulgare* L., *Echium webbii* Coincy, and *E. wildpretii* Pears. ex Hook. f.) was obtained from the Botanical Garden of the Vienna University, and of *Esterhazya* from private collections (St. Vogel). For investigations by Scanning Electron Microscopy (SEM) the *Echium* material was treated according to Halbritter (1998), for investigations by Transmission Electron Microscopy (TEM) according to Halbritter et al. (1997) and Weber et al. (1998), respectively. For Light Microscopy, anthers of *Esterhazya splendida* Mikan var. *angustifolia* were conventionally embedded, sectioned and stained with Astra Blue and Safranin.

Two different types of thread-shaped structures may be classified as: pollen-connecting threads, and cellular threads involved in pollen presentation.

In the first part we will consider the nature, occurrence and putative function of the various thread- or rope like structures, respectively; the second part is devoted to a conspectus on the respective origin.

Pollen-connecting threads

Acetolysis-resistant, sporopollenin threads

Occurrence. Acetolysis-resistant threads ("viscin threads") are rare and may be regarded as restricted to angiosperms. They are not only derived from the ektexine, but are in fact part of the ektexine itself, and thus their ornamentation is often quite similar to the ektexine sculpturing. Viscin threads occur in almost all recent Onagraceae and Ericaceae-Rhododendroideae (Skvarla et al. 1975, Skvarla et al. 1978, Waha 1984). The more prominent and more numerous viscin threads of fossil Onagraceae are well known (Keri and Zetter

1992). Nixon and Crepet (1993) and Zetter and Hesse (1996) presented reports on fossil Ericaceae pollen with viscin threads. Crepet (1996), however, assigns Turonian pollen material with viscin threads to a taxon of an ericalean/thealean complex. The so-called exinal connections in *Jacqueshuberia*, Caesalpiniaceae, are chemically, structurally, and most probably functionally very closely related to viscin threads (Hesse 1986, Patel et al. 1985). There is no substantive reason not to call the "exinal connections" viscin threads. In contrast to the viscin threads, the "exine bridges" in some Onagraceae (Skvarla et al. 1975) represent very short acetolysis-resistant fibers connecting individual monads within a permanent pollen tetrad. The extremely short length of these fibers, their occurrence only between neighbouring monads and their being formed not only by ektexine material are good reasons to distinguish them from viscin threads. Because of this and also because of where they occur, the interpretation that their phylogenetic or functional predecessors were long viscin threads is questionable. They may represent only a special case of the manifold compound pollen types (Knox and McConchie 1986).

Are viscin thread-like structures restricted to the angiosperms? There are only a few reports on poorly understood ropy or fibrous features in (fossil) gymnosperms and also (recent) ferns. Kurmann and Zavada (1994) mention the common occurrence of proximal exine filaments in the dispersed fossil (gymnosperm) pollen genus *Triadispora*, which allows for adhesion of individual pollen grains in a tetrad, and Trevisan (1971) writes of *Dicheiropollis*: "mature pollen grains were connected in pairs by two bundles of delicate strings". Such pollen connecting filaments are sometimes present in *Ginkgo biloba* (Kurmann and Zavada 1994). Whether (acetolysis-resistant?) perispore strands of some fern spores have any function is still not clear (Tryon 1986).

Function. Any pollen material with viscin threads points to a highly specialized pollination mode (Crepet 1996, Hesse 1986, Waha

1984, Nixon and Crepet 1993, Zetter and Hesse 1996). In Rhododendroideae, which are pollinated by bumblebees, honeybees, birds, and, to some degree, also by flies, the viscin threads are restricted to taxa with erect flowers (Wallace 1975) and are much thinner and more rare than in Onagraceae (Skvarla et al. 1978, Waha 1984). It should be stressed that pollenkitt is formed in taxa with viscin threads, but the mature pollen grains or tetrads in *Rhododendron*, *Jacqueshuberia*, and also in Onagraceae are nearly free of pollen coatings (Halbritter unpubl.). From this it can be speculated that the viscin threads dominate by far over pollenkitt as the vector in forming pollen aggregates. Most authors think that the only function of viscin threads is to form large, flexible pollen aggregates during the pollen transfer. In contrast, according to Rowley (1987, also for review) the primary function of viscin threads may not be to form large pollen aggregates but rather to fix pollen at the border of the opened pollen sac, to prevent pollen from falling off prematurely (they may even be interpreted as communication structures, Rowley 1987). The viscin threads would also play a role in pollen presentation. According to King and Buchmann (1995), however, long viscin threads with an average length of 9.5 mm were found adhering to the anther apical pore tips, hanging in the bee's pathway so that they draped over the back of a bee's thorax. The low frequency vibration modes of the viscin threads may contribute to the pollen ejection by low frequency stamen vibration. The viscin threads did not break apart even under the most vigorous vibration in a shaker, which points toward an astonishing flexibility and simultaneous stability of these seemingly fragile viscin threads.

In Onagraceae pollination often takes place by long-tongued insects (Willemstein 1987) or birds (*Fuchsia*). The heavy and robust flower-visiting animals may require a greater viscin thread strength, thus the thick, often complex and generally sculptured viscin threads of Onagraceae should have more tensile strength ("stiffness") than their thin, smooth counter-

parts in the Rhododendroideae. It should be noted that all viscin threads are highly flexible, but not elastic like a rubber band.

May we draw conclusions from fossil Rhododendroideae and Onagraceae findings to the pollination ecology in their time? All features point towards a highly specific plant-pollinator relationship and a pollinator spectrum in the Tertiary or even in the Cretaceous similar to the recent one. However, this advanced pollination syndrome using viscin threads as pollen connecting agent was established at least in the Eocene (Zetter and Hesse 1996), or even earlier (Upper Cretaceous: Crepet 1996, also for review): such a specific pollination syndrome could even be plausible for the middle and early upper part of the Cretaceous.

Sporopolleninous strands may appear also in some orchids. These strands differ from the genuine viscin threads in their mode of origin, and they probably also have a different function. Burns-Balogh and Funk (1986) described a sporopolleninous layer termed "cohesion strands" in pollinia (group of tetrads) of Spiranthoideae (*Schiedeella*). This layer is the cementing substance of the tetrads and is resistant to acetolysis. It assumes a thread-like appearance only when the tetrads are torn apart. The cohesion strands should not be confused with the viscin threads found in Onagraceae, because – as stated above – viscin threads are preformed filaments of sporopollenin and are extensions of the exine. The cohesion strands may act as reinforcement of soft pollinia (Dressler 1993). Ackerman and Williams (1981) reported earlier that acetolysis-resistant straplike bands connect sister grains within the tetrads of some *Caladenia* and *Chloraea* species. Concerning the possible role of viscin threads in relation to viability and desiccation see Dafni and Firmage (this volume).

Pollen-connecting threads not consisting of sporopollenin

They are found much more frequent than the rare sporopollenin-containing threads and have been in several angiosperm families.

Occurrence includes Annonaceae: *Porcelia* (Morawetz and Waha 1991); the slimy strands wrapping pollen grains in some Araceae (Richter 1929, Troll 1928). Aristolochiaceae: *Aristolochia* (M. Wolter: pers. comm.); Asclepiadaceae: *Mondia* (H. Kunze, Minden: pers. comm.), *Raphionacme* (Dannenbaum and Schill 1991); Caesalpiniaceae: *Bauhinia*, *Caesalpinia*, *Cercis*, and *Delonix* (all Hesse 1986); Heliconiaceae: *Heliconia* (Rose and Barthlott 1995); Hydrocharitaceae: *Halophila* and *Thalassia* (Cox and Tomlinson 1988, Pettitt 1981); Marcgraviaceae: *Norantea* (Pinheiro et al. 1995, Sazima et al. 1995); Orchidaceae: *Cypripedium* (Burns-Balogh and Hesse 1988), *Disa* (Vogel 1959), *Doritis* (Wolter et al. 1988), *Habenaria* (Hesse and Burns-Balogh 1984), *Zeuxine* (Shukla 1984, Vijayaraghavan and Shukla 1980), orchids in general (cf. Schill and Wolter 1986, and Dressler 1993); Passifloraceae: *Tetrastylis* (Buzato and Franco 1992); Strelitziaceae: *Strelitzia* (Kronestedt-Robards 1996), Zannichelliaceae: *Lepilaena* (Cox and Knox 1989).

Many Araceae genera with relatively smooth pollen extrude strands composed of pollen grains glued together (Mayo et al. 1997); it is unknown whether pollenkitt is the only glueing vehicle. These strands adhere to insect bodies, sometimes with the aid of sticky secretions within the inflorescence (Mayo et al. 1997). The Araceae are highly diverse: e.g. in *Calla* and *Anthurium*, where anthers are embedded inside the basal part of the inflorescence, the relatively sculptured (not smooth!) pollen grains are kept together by highly viscous pollenkitt (Pacini, Halbritter, pers. observations). In contrast, e.g. in *Arum*, the spiny pollen grains usually form a loose, powdery mass at the bottom of the trap. Another, quite different example for “pollen strands” is found in *Disperis* (Orchidaceae): the massulae appear glued in a regular manner on a long, elastic axial band, which is made from tapetal and sporogenous derivates (for details see Vogel 1959).

Function. Whereas pollen-connecting sporopollenin threads are found in almost all taxa of Onagraceae and Rhododendroideae, the occurrence of pollen-connecting thread-form-

ing material lacking sporopollenin is generally restricted to a single genus or few genera within the above mentioned families. This fact, but also the apparent exceptions of Orchidaceae (Dressler 1993, Schill and Wolter 1986) and Araceae (Mayo et al. 1997), point towards a respective highly specialized pollination syndrome. In practically all cases of sporopollenin-less threads, most pollen grains of an anther get entangled or – as in *Impatiens*, *Norantea*, and *Porcelia* – the entire pollen mass is surrounded and interspersed. This is not the case in *Gymnocalycium* where only a few pollen grains are connected by threads. In this respect *Gymnocalycium* differs significantly from all other thread-forming taxa, and this fact brings us to the next chapter.

Thread-shaped cellular structures involved in pollen presentation

Occurrence. Anther-borne threads may not only function to entangle individual grains forming pollen clumps. A “first step” in this direction is the case of the Cactaceae *Gymnocalycium* (Halbritter et al. 1997), because only a few pollen grains get entangled, and this happens incidentally (see below at the end of this article). Their occurrence in *Impatiens* (Balsaminaceae, Vogel and Cocucci 1988) is well known, but corresponding similar features have also been found in *Echium* (Boraginaceae) and *Esterhazyia* (Scrophulariaceae, Rhinanthoideae-Gerardieae).

The case of *Echium* (H. Halbritter)

In all investigated *Echium* species (*E. auberianum* Webb et Berth., *E. russicum* J. F. Gmelin, *E. cf. virescens* DC., *E. vulgare* L., *Echium webbii* Coincy, and *E. wildpretii* Pears. ex Hook. f.) cellular hairs or threads were found forming a basket over the open pollen sacs (Fig. 1e). This feature is probably found throughout the genus *Echium*. The cellular hairs are preformed in the still closed anther forming a curtain-like structure between the two pollen sacs (Fig. 2a, b). When the stomium

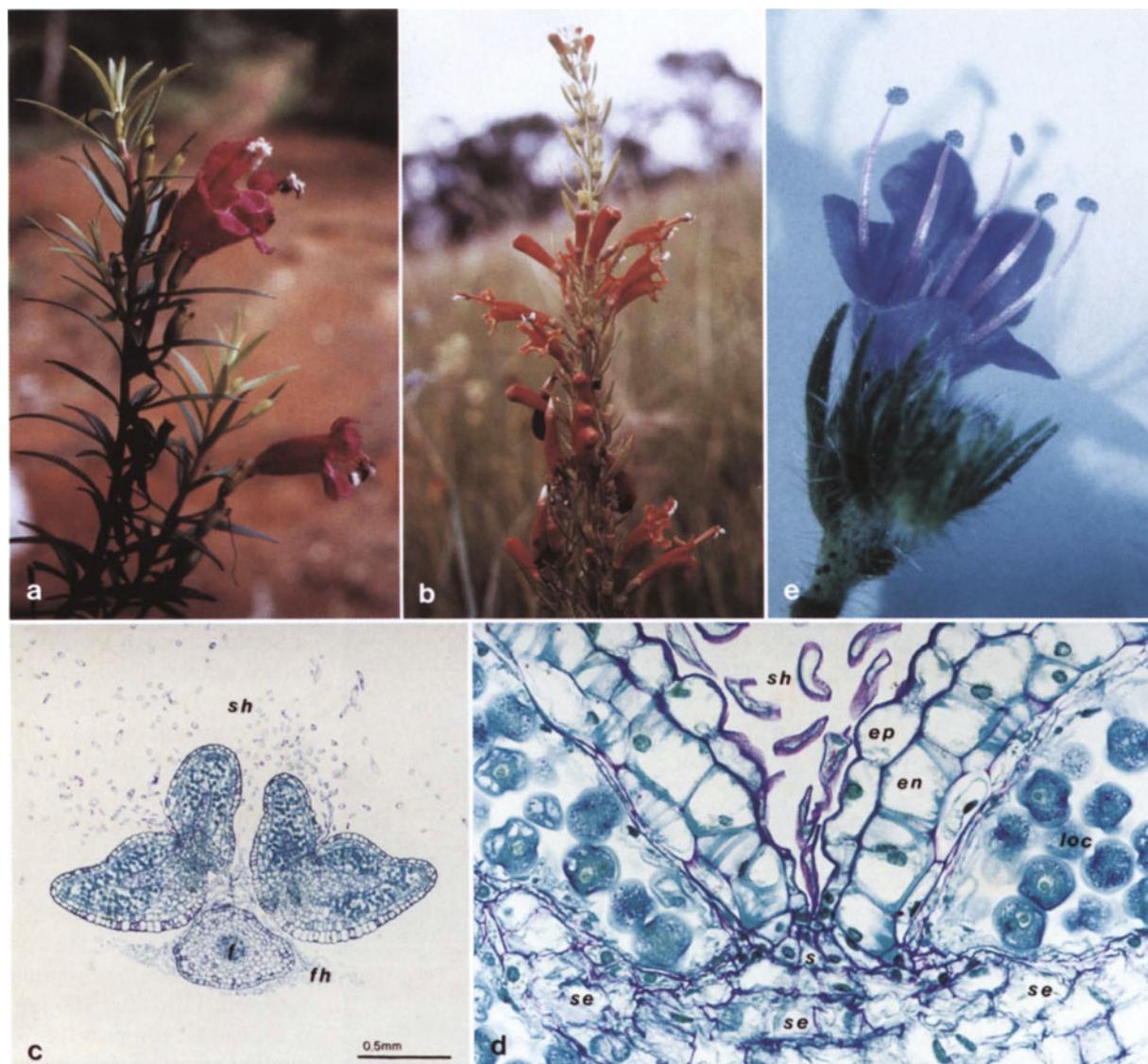


Fig. 1. **a** *Esterhazya splendida* var. *angustifolia* (Itatiaia, Brazil), **b** *Esterhazya* sp. (Paraná, Brazil), **c-d** *Esterhazya splendida*, anther in cross sections (stained with astra blue and safranin): **c** total, **d** stomial region of the theca. **e** *Echium vulgare*, flower with freshly opened anthers. *en* endothecium, *ep* epidermis, *f* filament, *fh* multicellular filament hairs, *loc* loculum, *s* stomium, *se* locular septum, *sh* unicellular stomial hairs. Bar = 0.5 mm

region opens, these hairs are visible spreading over the stomial slit (Fig. 2c, d). The slit gets enlarged and the locular walls start to bend outwards. During this movement the pollen is pressed through a basket of hairs spread between stomium and septum. Finally the pollen masses stick inside as well as outside on the threads and are held by this basket,

which covers the contorted pollen sacs completely. At this time the threads are hardly or not visible from the outside, being covered by pollen masses (Fig. 1e). After the pollen is removed, the remaining basket is visible (Fig. 2e). The primary and dominating function of the *Echium* anther hairs probably is not only to hinder the pollen from falling out

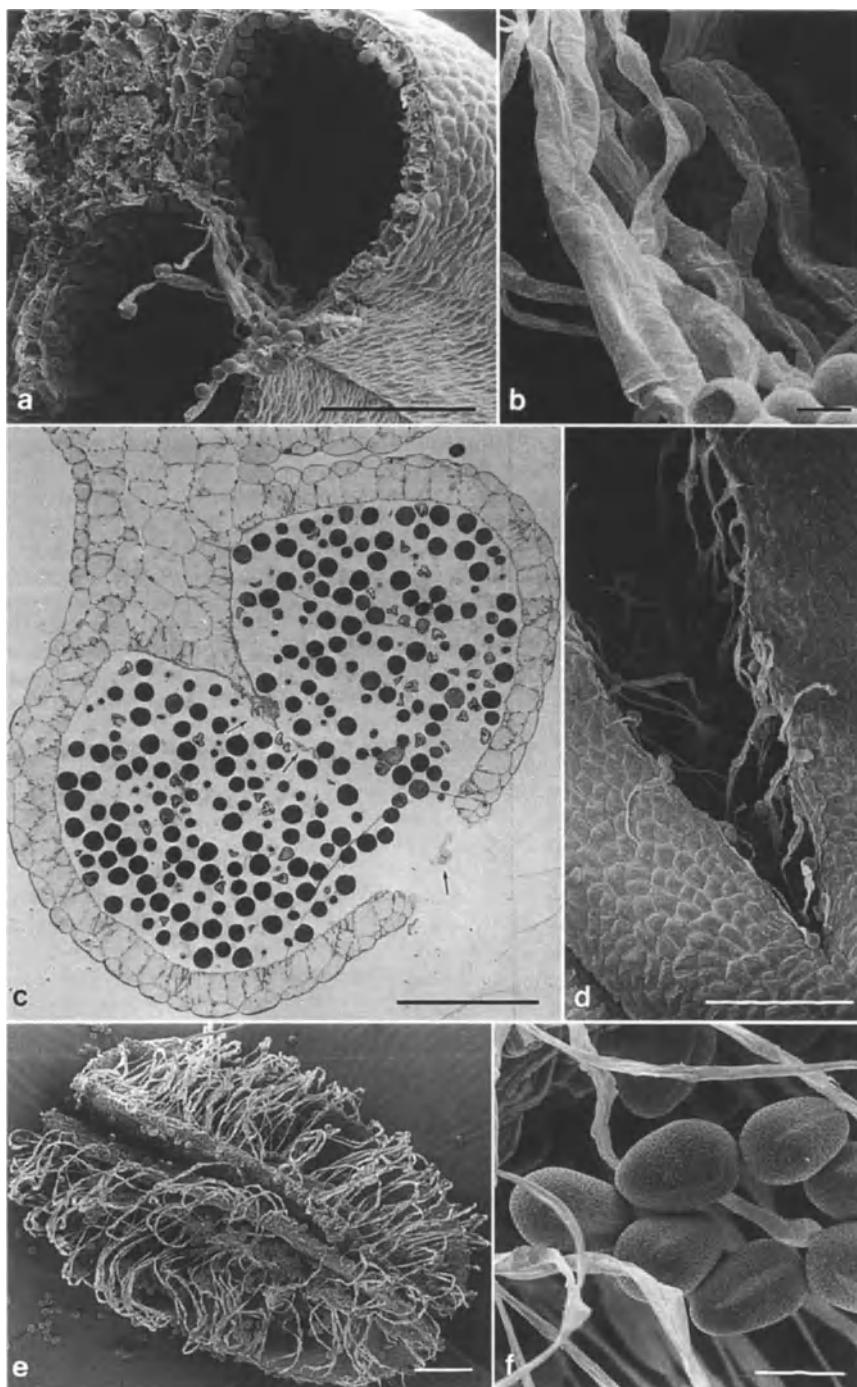


Fig. 2. **a-f** *Echium* sp.
a Section through the still closed anther of *Echium* cf. *virescens*. The septum is formed by curtain-like cellular hairs. SEM. Bar 100 µm.
b Detail of Fig. 2a. SEM. Bar 10 µm.
c *Echium vulgare*, cross section of an already slightly dehiscent anther. Note cellular elements in the area of the former stomium and septum (arrows). TEM. Bar 100 µm.
d *Echium* cf. *virescens*, beginning of anther dehiscence. SEM. Bar 100 µm.
e Completely opened anther of *Echium vulgare*, the major part of pollen is already removed. SEM. Bar 100 µm.
f *Echium auberianum* pollen grains sticking to some anther hairs. SEM. Bar 10 µm

prematurely, but also to partition and to present the blue-coloured pollen to flower visitors. The mature pollen is rather dry, and pollenkitt is found predominantly in the exine arcades of the pollen grains. Transmission electron micrographs of *E. vulgare* and scan-

ning electron micrographs of all investigated *Echium* species show rather “clean” pollen surfaces, and only a few grains stick to the threads (Fig. 2f). In just opened pollen sacs, sections of cellular threads were found not only near the stomium (epidermal cells), but

also in positions formerly held by the septum (Fig. 2c), which contain cytoplasmic and lipid remnants. Additionally, some lipid material (from the former tapetal cells, but perhaps also from degenerated stomium cells and, more probably, from septal cells) adhere to the hairs, making them sticky.

The *Echium* pollen baskets are reported for the first time. Surprisingly, all former investigators did not take notice of this feature (e.g. Kraemer and Schmitt 1997). The hairs are not pollen-connecting threads in sense of viscin threads, but of course play a role in pollination ecology of *Echium*. Functionally they are comparable to the *Impatiens* (Balsaminaceae) basket equipment. The *Impatiens* fibers perhaps act as a “net”, protecting pollen grains from escaping “too early”, but also connect individual grains, which are somewhat sticky.

In *Impatiens* and *Echium*, cellular elements of the anther's interior give rise to a net that spans the open thecae to prevent the pollen from falling off prematurely. However, *Echium* threads are not formed as in *Gymnocalycium*. The *Gymnocalycium* case study thus cannot be compared with *Echium*, because in *Gymnocalycium* a pollen basket is lacking, and only some pollen grains get entangled *per incident*. The following example demonstrates that a similar effect may be achieved in an analogous manner by trichomes of the epidermal layer of the thecal wall.

Usually, thecal walls are bald. Occurrence of trichomes on their epidermis is infrequent. For instance, anthers of certain Magnoliidae (*Balanops*, *Calycanthus*) and Lower Rosidae (*Amphipterygium*, *Koelreutheria*) bear short trichomes loosely distributed over the whole thecal surface, as depicted by Endress and Hufford (1989) and by Endress and Stumpf (1991), respectively. Among the Scrophulariales there are more pronounced thecal indumenta giving the anthers a villose or bearded appearance as in *Amphicoma* (Bignoniaceae) and species of *Gerardia* (Scrophulariaceae) and several other genera of the Rhinantoideae (e.g. *Bartsia*, *Rhinanthus*, *Lathraea*, Knuth 1899,

p. 176 ff.). In these cases there is little evidence thus far for any function connected with the release of pollen. In *Acanthus* (Acanthaceae) conspicuous “brushes” of hairs derive from at first stages compact and dense, but at later stages split modified septal cells (cf. Knuth 1899, vol. 2/2). In *Lathraea*, hair rows prevent the pollen from being dispersed in the lateral, i.e. inefficient, direction (Knuth 1899). In *Euphrasia*, stomial tufts of crisp hairs serve to connect neighbouring anthers, which bear corresponding counterparts, to form a functional unit (Hartl 1972).

The case of Esterhazya (S. Vogel)

In the genus *Esterhazya*, from which *E. splendida* Mikan var. *angustifolia* was investigated, such thecal hairs are obviously specialized to retain and harbour the pollen following anther dehiscence and to partition its release. The anthers, measuring 3.5–4.7 mm in length, are extremely bearded (Fig. 1a–d, Fig. 3a, b). The trichomes of this beard are strictly confined to the longicidal furrows of the thecae, where they are inserted in two single, dense rows flanking the dehiscence suture along its entire length (Fig. 3c). The stomium in between consists of small-celled tissue that joins the massive septum with the thecal walls. The hairs are unicellular, unbranched (sometimes basally bifurcate), undulate in shape, rather stiff, with a rugged cuticle, and somewhat flattened already in their lifetime and more so when they dry up at maturity (Fig. 1c, d, Fig. 3d). All of them emerge obliquely from the epidermis, converging towards the proximal end of the theca. Due to their crisp condition, the two adjacent rows of hairs interweave soon after their early formation in the bud (similar hairs are initiated at the stage of meiosis in the allied genus *Agalinis*, Canne-Hilliker 1987). Because they do not disentangle but only become spread at the time of anther dehiscence, they form, together with the beard of the adjacent theca, a layer of loose, whitish felt that is as voluminous as the anther itself and finally covers the aperture of each theca.

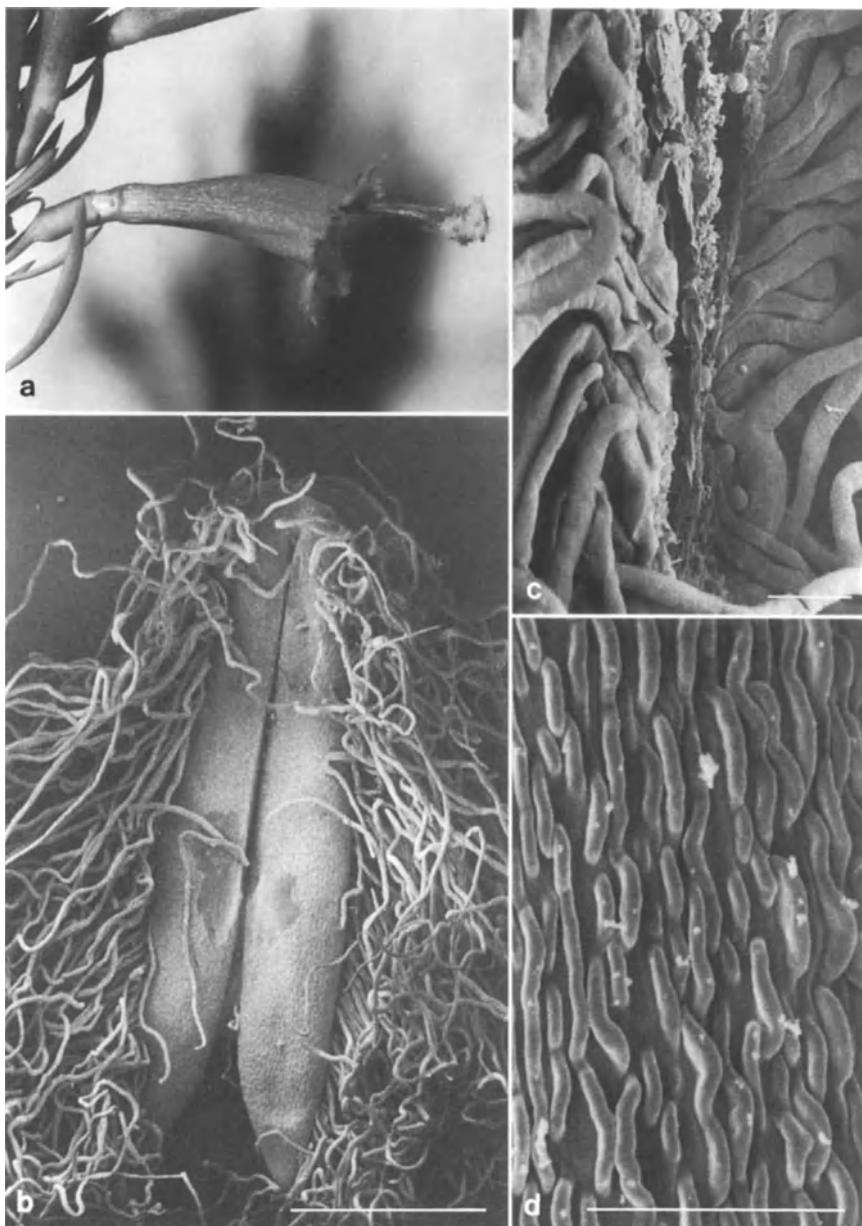


Fig. 3. a–d *Esterhazyia splendida*. **a** Flower ($\times 1.2$), showing the voluminous hair puff formed by the four anthers. **b** Anther, ventral view; the tiers of unicellular trichomes line the stomial sutures of the two (still undehisced) thecae. SEM. Bar 1 mm. **c** Insertion of the hair tiers along either side of the stomial suture of one theca, which is beginning to split (above). SEM. Bar 100 μ m. **d** Surface of a stomial hair. SEM. Bar 10 μ m

The four closely adjacent anthers face downward in the horizontally oriented flower, providing nototribic pollination. Without any protection, their content of rather dry, powdery pollen would easily fall off prematurely and as a whole – a waste that is obviously prevented by the cover of hairs. It retains the grains until they can be released in small portions upon friction of the anthers during repeated visits of pollinators. Thus the hair felt functions like a powder-puff. The parts dusted

are the forehead or crown of birds. Two unidentified species of hummingbirds have been observed repeatedly by one of the authors (S. V.), when they were foraging nectar from these ornithophilous flowers in the State of Paraná, Southern Brazil.

Beck v. Mannagetta (1895) has already recognized this function when attributing it to the anthers of Orobanchaceae: “Trichomes which often line the dehiscence cleft of the anthers prevent unsuitable dispersal of the

pollen" (see also Knuth 1899). These hairs, however, are in the *Orobanche* species we examined very short and do not span the aperture. Species of *Agalinis* (same tribe as *Esterhazya*) and *Lamourouxia* (Rhinanthoideae-Rhinantheae) display similar powder-puffs as we have described for *Esterhazya*.

In *Esterhazya* the distal part of the filament and the connective are also strongly villous, forming a tangled indumentum similar in appearance and closely adjacent to the thecal beard. Its trichomes, however, differ from the thecal trichomes in being multicellular, more thin-walled, still alive at anthesis, and with vacuoles containing purple anthocyanine: Their function, if any, is unknown.

Conspectus on nature and mode of origin of the thread-forming structures

Although the nature of sporopollenin-consisting threads is well-known, our knowledge about the mode of origin is very meagre. Rowley (1987) associates viscin threads with plasmodesmata processes. However, Keri and Zetter (1992) found cytomictic channels between the individual tetrad members in young *Epilobium* tetrads. If the channel material consists of primexine matrix, then (during the callose dissolution and during tetrad member separation) sporopollenin will be incorporated as is typical in ektexine formation. Thus the (permanent?) cytomictic channels in the callose wall between the young tetrad members may be the starting point of viscin threads. If cytomictic channels would exist also between the tetrads, then this model for viscin thread formation could be valid also for the many permanent, viscin-thread-connected tetrads in Rhododendroideae and Onagraceae. The nature and mode of origin of sporopollenin-less threads, which are known to arise in various ways, are much better known (Halbritter et al. 1997). Five modes of origin have been recognized to date. (A) Ordinary pollenkitt may sometimes assume a rope-like habit (Halbritter and Hesse, pers. observations). The pollenkitt viscosity probably depends on its specific

chemical composition: pollenkitt composed predominantly of unsaturated lipids with a higher number of bonds is of higher viscosity than pollenkitt mainly composed of saturated lipids (Pacini and coll., unpubl.). (B) In some unrelated angiosperm taxa, as in *Porcelia* (Annonaceae), in *Aristolochia*, and in all mentioned Caesalpiniaceae, a slimy mixture of lipids and cytoplasmic components (both most probably deriving from tapetal cells only) may form thread-like structures. (C) In other angiosperm taxa, such as in *Raphionacme* (Asclepiadaceae) and in many Orchidaceae, the "elastoviscin" is composed of lipid products of the tapetal cells' ground plasm and endoplasmic reticulum (ER) only (Dannenbaum and Schill 1991, Dressler 1993, Wolter et al. 1988). Elastoviscin is regarded as homologous to pollenkitt: It occurs between pollen tetrads or pollinia and their appendicular structures as a clear, highly viscous and elastic substance (Schill and Wolter 1986). Either it holds the orchid pollinia together (Dressler 1993), or it may act as its own viscidium (Burns-Balogh and Hesse 1988). Elastoviscin assumes a thread-like appearance only when pollen grains are physically separated. (D) In other orchids cellular and/or sporopollenin parts of the caudicle and pollen-forming tissues may be involved in the formation of thread-forming elastoviscin (*Disa*, Vogel 1959; *Habenaria*, Hesse and Burns-Balogh 1984). (E) A quite different manner of thread formation occurs in *Gymnocalycium*, *Heliconia*, *Impatiens*, and *Strelitzia*. Their long and robust threads derive from cells along or near the stomial dehiscence line, and, contrary to (A–D), they are formed before pollen is physically separated. These threads are either of cellular origin, as in *Strelitzia*, or only parts of distinct cells, as in *Impatiens* and in *Heliconia*. In *Gymnocalycium*, and likewise in *Echium* and *Esterhazya*, they are made up of modified stomial and/or septal cell walls together with distinct derivates of tapetal cells. However, these threads have significantly different functions. In *Heliconia* and *Strelitzia* they act as a pollen clumping agent. In

Gymnocalycium the few threads entangle a few pollen grains only by accident, the majority of pollen is not involved, and thus no direct function in pollination ecology is given as in *Echium* and *Esterhazya*, where pollen baskets are formed. One may speculate that *Gymnocalycium* represent first steps towards a pollen basket.

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Convergent evolution and adaptive radiation of beetle-pollinated angiosperms

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Abstract. A literature review of 34 families of flowering plants containing at least one species pollinated primarily by beetles is presented. While the majority of species are represented by magnoliids and basal monocotyledons specialized, beetle-pollinated systems have evolved independently in 14 families of eudicotyledons and six families of petaloid monocots. Four, overlapping modes of floral presentation in plants pollinated exclusively by beetles (Bilabiate, Brush, Chamber Blossom and Painted Bowl) are described. Chamber Blossoms and Painted Bowls are the two most common modes. Chamber Blossoms, found in magnoliids, primitive monocotyledons and in some families of woody eudicots, exploit the greatest diversity of beetle pollinators. Painted Bowls are restricted to petaloid monocots and a few families of eudicots dependent primarily on hairy species of Scarabaeidae as pollen vectors. In contrast, generalist flowers pollinated by a combination of beetles and other animals are recorded in 22 families. Generalist systems are more likely to secrete nectar and exploit four beetle families absent in specialist flowers. Centers of diversity for species with specialized, beetle-pollinated systems are distributed through the wet tropics (centers for Brush and Chamber Blossoms) to warm temperate-Mediterranean zones (centers for Painted Bowls and a few Bilabiate flowers). It is unlikely that beetles were the first pollinators of angiosperms but specialized, beetle-pollinated flowers must have evolved by the mid-late Cretaceous to join pre-existing guilds of beetle-

pollinated gymnosperms. The floras of Australia and western North America suggest that mutualistic interactions between beetles and flowers has been a continuous and labile trend in angiosperms with novel interactions evolving through the Tertiary.

Key words: Chamber Blossom, chewing mouth-parts, *Coleoptera*, Cretaceous, Painted Bowl, magnoliids, monocotyledons (basal *vs.* petaloid), Scarabaeidae.

An in depth, inclusive review on beetle-pollination seems long overdue. Scholarly publications detailing the role of *Coleoptera* as pollen vectors have been available since the second half of the nineteenth century. Historians of Botany often credit Hermann Muller as the first antheconomist to recognize two traits intrinsic to the floral syndrome he called *Cantharophila*. First, beetles that pollinate flowers must have mouth-parts modified for the collection and consumption of pollen and/or nectar. Second, flowers that are dependent on beetles as pollen vectors offer a predictable suite of interlocking characters differing significantly from flowers pollinated primarily by insects belonging to other Orders (see Knuth 1906, Barth 1985).

During the twentieth century the study of beetle-pollination was co-opted by evolutionary botanists after Diels (1916) noted that

some *Coleoptera* pollinated flowering plants considered primitive by some taxonomists. Beetle-pollinated flowers became important model systems essential for studies on the origin and evolution of angiosperms (Gottberger 1977, Bernhardt and Thien 1987, Endress 1987, 1990, Takhtajan 1991). The high proportion of beetle-pollinated systems within magnoliid relictus was influential in maintaining a long-standing theory that modern flowers were derived largely from beetle-pollinated protoangiosperms (Eames 1961, Richards 1986, Crepet and Friis 1987). This explains why, for more than 80 years, magnoliid flowers have been the “privileged pets” of so many paleobotanists, palynologists, and developmental botanists devoted to the study of the origins of floral structures (Eames 1961, Takhtajan 1991, Endress 1990, 1994).

However, the importance attributed to beetle-pollination within evolutionary botany never ensured that this floral syndrome received more fieldwork than those taxa pollinated by air currents or other animal vectors. Quite the contrary, observations and experiments on cantharophilous flowers received only sporadic attention during the twentieth century. Botanists were often unfamiliar with significant contributions written by entomologists (see Peringuey 1902). Therefore, when beetle-pollination was reviewed botanical authors almost invariably devoted less space to it than to other insect-pollinated systems. Most reviewers offered the same excuse for unbalanced critical treatments. That is, beetle-pollination is uncommon in temperate zones and tropical pollination ecology lags behind fieldwork in temperate regions of the Northern Hemisphere. Therefore, a definitive review of cantharophily must wait for significant advances in tropical research (Knuth 1906, Fægri and van der Pijl 1979, Gottsberger 1977, Barth 1985, Proctor et al. 1996).

We can no longer afford to wait for a “Golden Age of Tropical Research” to assess the extent of diversification of beetle-pollination in angiosperms. As tropical floras are undergoing a swift decline it is most unlikely

that beetle-pollinated species will prove to be more resilient to human depredations (Allen-Wardell et al. 1998). Also, within the past 20 years additional publications on beetle pollination have accumulated to the point that there is now more than enough disparate literature available to interpret an unusually sophisticated syndrome. Furthermore Farrell (1998) offers convincing evidence that diversification within the lineage of phytophagous beetles follows the origin and diversification of angiosperms. If predation on seed plant structures has encouraged diversification within the Order *Coleoptera* it's time to consider what evolutionary trends may indicate about the diversity and convergence of flowers that recruit beetles as pollinators. Unfortunately a recent, paleontological treatment (Grimaldi 1999) excludes beetle fossils within a comparative study of the early evolution of flower-visiting insects (Grimaldi 1999). It is time to ask the question whether pollen presentation and pollen reception mechanisms exist that are unique to beetle-pollinated flowers?

Restraints to the study of cantharophily

In all fairness, it is often difficult to complete and interpret field projects related to cantharophily. From the botanical perspective, access to geographic centers of beetle-pollination is often limited due to relative distance and topography (Table 1). The sheer height of mature, tropical trees and woody vines is often intimidating while the lifespan of individual, magnoliid flowers is notoriously brief (Primack 1985). Some “blossoms” consist either of many, small, sexual organs surrounded by a stiff, oversized perianth or reduced, unisexual flowers enveloped by an inflated bract (Table 2). Consequently, once the insect enters the floral chamber it is lost from view unless the blossom is picked and opened manually. This destructive action ruins future opportunities for experimental manipulations and further visits by insects.

From the entomological perspective, the taxonomy of most *Coleoptera* is so poorly

Table 1. Proportion of Taxa with Beetle-Pollinated systems (Specialized and Generalized) In Different Biomes. (*AM* arborescent monocots (palms); *AS* shrubby Asteraceae; *CT* canopy trees; *GH* geophytic herb; *E* epiphytes; *HM* diverse, herbs (including geophytes, epiphytes and/or vines); *L* lianes; *PS* shrubby Proteaceae; *S* diverse shrubs; *SCT* subcanopy trees)

Geography/Ecosystem	Life Form(s) sampled*	% of Taxa Pollinated by Beetles	References
Mediterranean Israel Garrigue and Phrygana	CT, GH, S	0.0025	Dafni and O'Toole 1994
Indomalaysia Dipterocarp Forest	CT, E, GH, L, SCT	20.00	Momose et al. 1998
Mesoamerica Lowland rainforest	CT SCT	0.00 10.70	Bawa et al. 1985 Bawa et al. 1985
South Africa Fynbos karroid shrubland and sandplain fynbos	PS GH, AS	15.38 33.96	Collins and Rebelo 1985 Picker and Midgley 1996
South America Cloud Forest Palm Swamp	AM, H GH, H	45.10 2.50	Seres and Ramirez 1995 Nelson and Ysaleny 1998

understood that it is often impossible to identify flower-visiting specimens beyond the level of subfamily (Bernhardt 1989). Beetle behavior is often uncooperative as many of these insects are nocturnal (Baker and Baker 1990, Rodrigues et al. 1993) making direct observation difficult, expensive and dangerous. After entering a floral chamber a solitary beetle may not exit the blossom for hours taxing the budgets and schedules of potential observers.

This is problematic as Gottsberger (1988, 1989a, b) has shown conclusively that the diversity of flower beetles within a blossom does not imply that all visiting *Coleoptera* are pollinators. Without careful observation vandals and thieves may be misidentified as pollinators. Comparatively few pollination ecologists determine whether beetles actually transport the pollen of a host species while contacting receptive stigmas (Bernhardt 1976, Wallace 1980, Goldblatt et al. 1998). For example while scarabaeid beetles (Scarabaeidae sensu Boror et al. 1989) visit flowers of *Hibbertia spp.* well dusted with pollen of

myrtaceous taxa they are insignificant vectors of *Hibbertia* pollen and destroy organs within *Hibbertia* flowers (Hawkeswood 1989, 1992, 1993; Bernhardt 1996; Bernhardt and Tucker in progress).

In fact, the chewing mouth-parts of most flower beetles have perplexed both botanists and entomologists (see Grimaldi 1999) as some beetle species regularly combine the roles of floral predator and pollinator. This is such an old problem that Knuth (1906) remained uncertain as to the status of beetle visitors collected in flowers of domesticated and native species in Europe. The observations of Hawkeswood (1989, 1992, 1993) show plainly that when the flowering periods of different taxa overlap one beetle taxon may prey on the flowers of one genus while pollinating others. Bernhardt (1989) questioned the effectiveness of 31 beetle taxa as pollinators of nine *Acacia spp.* as the majority of beetles consumed whole carpels on the host inflorescence although they also transported their host flower's pollen. *Asclera ruficollis* (Oedemeridae) carries the

Table 2. Comparative Blossom Presentation (single flower or inflorescence) vs. Beetle Diversity in Angiosperms Pollinated Primarily by Coleoptera. Number in parenthesis refers to number of species examined. Floral Form (*B* Brush, *BL* Bilabiate, *C* Chamber Blossom, *PB* Painted Bowl); *BM* beetle marks (Optic Black), *SS* strongly scented; *EX* anthers extrude or shed pollen at anthesis; *ER* edible rewards (*fb* discrete food bodies, *n* nectar, *p* pollen; *pe* perianth segments; *ss* stigmatic secretions; *st* whole stamens or edible staminodia); Coleoptera Families (*Ant* Anthicidae, *Ceram* Cerambycidae, *Chr* Chrysomelidae, *Cler* Cleridae, *C* Curculionidae, *Elat* Elateridae, *Hyd* Hydrophilidae or Hydraenidae, *Lyc* Lycidae, *Mel* Melyridae, *Mor* Mordellidae, *Nit* Nitidulidae, *Oed* Oedemeridae, *Ptil* Ptiliidae, *Rhiz* Rhizophagidae, *Scar* Scarabaeidae, *Scrap* Scraptiidae, *Staph* Staphylinidae)

Plant Taxon	Floral Form	BM	SS	EX	ER	Coleoptera* Families
Annonaceae						
<i>Anaxagorea</i> (1)	C	-	+	+	pe	Nit, Staph
<i>Annona</i> (17)	C	-	+	+	p, pe	Ceram, Cur, Nit, Scar
Araceae						
<i>Asimina</i> (2)	C	-	+	+	n, p	Ceram, Scar
<i>Cathostema</i> (1)	C	-	+	+	ss	Cur
<i>Cymbopetalum</i> (2)	C	-	+	+	?	Scar
<i>Desmopsis</i> (1)	C	-	+	+	?	?
<i>Dugetia</i> (2)	C	-	+	+	p, pe	Cur, Nit
<i>Enciosanthum</i> (2)	C	-	+	+	pe, ss	Chry, Cur, Scar
<i>Fissistigma</i> (1)	C	-	+	+	ss	Cur
<i>Friesodielsia</i> (2)	C	-	+	+	ss	Cur, Nit
<i>Goniothalamus</i> (3)	C	-	+	+	ss	Cur, Nit
<i>Guatteria</i> (8)	C	-	+	+	p	Cur?, Nit
<i>Meiogyne</i> (1)	C	-	+	+	ss	Cur, Nit
<i>Monocarpia</i> (1)	C	-	+	+	ss	Cur, Nit
<i>Polyathia</i> (6)	C	-	+	+	pe, ss	Chry, Cur
<i>Porcelia</i> (1)	C	-	+	+	p, pe	Cur, Nit
<i>Pyramidalthe</i> (1)	C	-	+	+	ss	Cur
<i>Rollinia</i> (2)	C	-	+	+	p, pe	Nit
<i>Uvaria</i> (1)	C	-	+	+	ss	Cur
<i>Xylopia</i> (1)	C	-	+	+	p, pe	Cur, Nit
Arecaceae						
<i>Amorphophallus</i> (2)	C	-	+	+	fb, p, pe	Nit
<i>Dffenbachia</i> (2)	C	-	+	+	st	Scar
<i>Homalomena</i> (1)	C	-	?	?	p, st	Chry, Scar
<i>Montrichardia</i> (1)	C	-	?	?	pe	Scar
<i>Philodendron</i> (3)	C	-	+	+	pe	Scar
<i>Typhonium</i> (1)	C	-	+	+	-	Scar
<i>Xanthosoma</i> (1)	C	-	+	+	pe	Scar
Bombacaceae						
<i>Astrocaryum</i> (1)	B	-	?	?	?	?
<i>Bactris</i> (5)	B	-	+	-	p, pe	Cur, Nit, Scar, Staph
<i>Cryosophila</i> (1)	B	-	+	-	pe	Cur, Nit Scar, Staph
<i>Hydriastele</i> (1)	B	-	+	+	pe	Cur
<i>Coelostegia</i> (1)	C	-	?	?	p	Chr, Elat

Table 2 (continued)

Plant Taxon	Floral Form	BM	SS	EX	ER	Coleoptera* Families
Calycanthaceae						
<i>Calycanthus</i> (1)	C	-	+	+	st, pe	Nit
Campanulaceae						
<i>Pristmatocarpus</i> (1)	PB	-	-	-	p	Scar
<i>Wahlenbergia</i> (1)	PB	+	-	-	n?, p	Scar
Clusiaceae						
<i>Clusia</i> (1)	C	-	+	+	p	Chry
<i>Garcinia</i> (1)	C	-	?	?	-	Chry, Cur
Convolvulaceae						
<i>Erycibe</i> (1)	C	-	?	?	p	Mord
Cornaceae						
<i>Mastixia</i> (1)	PB	-	?	?	n, p	Chry
Cyclanthaceae						
<i>Asplundia</i> (4)	C	-	?	?	p, pe	Cur, Hyd, Ptil
<i>Cyclanthus</i> (1)	C	-	+	-	pe	Scar
Degeneriaceae						
<i>Degeneria</i> (1)	C	-	+	+	st?	Nit
Dipterocarpaceae						
<i>Hopea</i> (1)	C	-	+	?	p, pe	Chry, Cur
<i>Shorea</i> (17)	C	-	+	?	n, p, pe	Chry, Cler, Cur, Nit
<i>Vatica</i> (2)	C	-	+	?	p, pe	Chry, Cur
Droseraceae						
<i>Drosera</i> (2)	PB	+	-	-	p	Scar
Ebenaceae						
<i>Diospyros</i> (1)	C	-	?	?	p	Nit, Staph
Eupomatiaceae						
<i>Eupomatis</i> (1)	C	-	+	+	st	Cur
Hyacinthaceae						
<i>Ornithogalum</i> (1)	PB	+	-	-	n, p	Scar
Hypoxidaceae						
<i>Spiloxene</i> (2)	PB	+ -	-	-	n, p	Scar
Icacinaceae						
<i>Iodes</i> (1)	PB	-	?	?	p	Chry, Nit
Iridaceae						
<i>Aristea</i> (3)	PB	+	-	-	p	Scar
<i>Homeria</i> (2)	PB	-	-	-	n, p	Scar
<i>Ixia</i> (4)	PB	+ -	-	-	n, p	Scar
<i>Moraea</i> (7)	PB	+	-	-	p n	Scar
<i>Romulea</i> (1)	PB	+	-	-	p	Scar
<i>Thereianthus</i> (1)	PB	-	-	-	p	Scar
<i>Tritonia</i> (3)	PB	+ -	-	-	n, p	Scar
Lauraceae						
<i>Ocotea</i> (1)	PB	-	?	?	n?	?
Liliaceae						
<i>Tulipa</i> (1)	PB	+	-	+	p	Scar
Lowiaceae						
<i>Orchidantha</i> (2)	BL	- +	+	-	-	Scar

Table 2 (continued)

Plant Taxon	Floral Form	BM	SS	EX	ER	Coleoptera* Families
Magnoliaceae						
<i>Magnolia</i> (10)	C	-	+	+	p, s, n?	Ceram, Chry, Cur, Mord, Nit, Oed, Scar, Scrap, Staph
<i>Talauma</i> (1)						
	C	+	+	+	pe	Scar
Monimiaceae						
<i>Tambourissa</i> (3)	BC	-	+ -	+	p, n?, ss?	Cur, Hyd, Rhiz, Staph
Myristicaceae						
<i>Gymnanthera</i> (1)	C	-	?	+	p	Chry, Cur
<i>Knema</i> (3)	C	-	?	+	p	Cur, Staph
<i>Myristica</i> (2)	C	-	+	+	p	Ant, Scar
Nymphaeaceae						
<i>Nuphar</i> (1)	C	-	+	+	p, n?	Chry, Scar
<i>Nymphaea</i> (3)	C	-	+	+	p, pe?	Scar
<i>Victoria</i> (1)	C	-	+	+	fb, p	Scar
Orchidaceae						
<i>Ceratandra</i> (1)	Bl	-	+	-	-	Scar
<i>Peristeranthus</i> (1)	Bl	- +	+	-	n?	Elat, Lyc
<i>Pteroglossaspis</i> (1)	BL	+	+	-	n	Scar
Papaveraceae						
<i>Papaver</i> (1)	PB	+	-	+	p	Scar
Piperaceae						
<i>Piper</i> (1)	B	+	?	+	p	Cur
Polemoniaceae						
<i>Ipomopsis</i> (1)	B	+ -	+	-	n, p	Mel
<i>Linanthus</i> (1)	C	-	?	-	n, p	Mel
Ranunculaceae						
<i>Anemone</i> (1)	PB	+	-	+	p	Scar
<i>Adonis</i> (1)	PB	+	-	+	p	Scar
<i>Ranunculus</i> (1)	PB	-	-	+	p	Scar
Sterculiaceae						
<i>Heritiera</i> (2)	C	-	?	?	p	Chry, Cur
<i>Sterculia</i> (2)	C	-	?	?	p	Chry
Tiliaceae						
<i>Luehea</i> (1)	PB	?	?	?	n	Scar, Cur
Winteraceae						
<i>Exospernum</i> (1)	C	-	+	+	p, pe	Cur
<i>Zygogynum</i> (1)	C	-	+	+	p, ss	Cur

pollen of *Hepatica acutiloba* but also destroys the flower's ovaries (Bernhardt 1976).

In contrast, Sakai et al. (1999) has shown that representatives of three beetle families are more effective as pollinators of *Shorea*

parvifolia (Dipterocarpaceae) than are thrips found in the same flowers. Furthermore, Gottsberger (1977) showed that a beetle taxon acts as both a floral predator and pollinator in the same flower. *Epicauta strigosa* (Meloidae) is

one of the few frequently observed foragers on flowers of *Calydorea coelestina* (Iridaceae). This beetle consumes the entire perianth and androecium of the flower but the undamaged, inferior ovary sets viable seed after the beetle departs (P. O'Hara, personal communication). Therefore, once we accept that there are deficiencies in the way data is collected and interpreted it is possible to subdivide and review the available literature with some care.

Diversity of beetle-pollinated flowers

While pollination systems may be divided into overlapping categories there are more than 184 angiosperm species that are pollinated almost exclusively by beetles subdividing into 85 genera representing 34 families. Representatives from fourteen, beetle families are associated with these flowers but >0.47 of the plant genera are visited partly, or exclusively, by members of the family, Scarabaeidae (Table 2).

In contrast over 98 species in 40 genera representing 22 families are pollinated by a combination of beetles and other animals (Table 3). That is, beetles comprise a predictable, but not necessarily the largest, or most efficient, portion of the pollinator vector spectrum (sensu van der Pijl and Dodson 1966) of a particular plant species. Representatives from nine families of beetles are found on these generalist flowers. It is interesting to note that generalist flowers of 6 genera (*Acacia*, *Baccharis*, *Baeckea*, *Bursaria*, *Illicium* and *Nelumbo*) attract species in four beetle families (Buprestidae, Cantharidae, Carabidae, and Coccinellidae) not associated with flowers pollinated almost exclusively by beetles (Tables 2, 3). Taxa in the Scarabaeidae were collected on 23 genera representing >0.57 of all species listed in Table 3. It is also noted that beetle species within the Nitidulidae, a common and putatively ancient family of beetle pollinators, are found on only one of these generalist species (*Drimys brasiliensis*, Table 3).

It is acknowledged that Table 3 would be much expanded if all generalist systems that

incorporate representatives of at least one beetle family were included by following the complete Appendix provided by Momose et al. (1998). This would add data on generalist systems in both magnoliids (Lauraceae and Piperaceae) and approximately 11 families of eudicots. Unfortunately some entries in this Appendix are so incomplete (e.g. note the absence of information on floral odor) they have been excluded from Table 3 to avoid biased speculations.

Shared characters of beetle-pollinated flowers

We turn first to general aspects regarding the diversity and habit of species pollinated primarily by beetles. As anticipated by earlier authorities, beetle-pollination is more common in the Class *Eudicotyledones* (sensu Raven et al. 1999) compared to the *Monocotyledones* (Table 2). With important exceptions, beetle-pollination is more common in angiosperm families with woody habits and has been documented most extensively within tropical-moist and Mediterranean or warm-temperate (mesic) biomes (Table 1).

The physical construction of most beetle-pollinated blossoms can be subdivided into two separate units. Regardless of unit, though, the vast majority of beetle-pollinated blossoms are usually large enough for several beetles to enter the same blossom at the same time. Therefore, the first unit consists of relatively large, usually bisexual, individual flowers. Their petals, tepals or floral receptacles form salver, bowl or urn-like shapes (e.g. *Annona*, *Magnolia*, *Moraea*, *Tulipa* etc.). The volume of each individual flower is great enough to accommodate several beetles within the same floral cup.

In contrast, the second unit consists of tiny, often unisexual florets that may flower synchronously. They are united together on a compact and/or compound branch. This flowering branch is either exposed on a terminal shoot (e.g. Arecaceae and Piperaceae) or is enveloped in part by an expansive bract or bracts (e.g. Araceae and Cyclanthaceae). Once

Table 3. Comparative Features of Plants Pollinated by Beetles and other animals. *Abbreviations as in Table 1 with the following exception (*MS* multistaminate presentation of individual flowers of whole inflorescences; Beetle Families, *Bruch* Bruchidae, *Bup* Buprestidae, *Car* Carabidae, *Can* Cantharidae; *Coc* Coccinellidae, *Derm* Dermistidae; *Harp* Harp; *Lag* Lagriidae; *Lyc* Lycidae. Other vectors, *AC* air currents, *AP* bees-eusocial Apidae, *BI* birds, *D* Diptera, *L* Lepidoptera, *M* mammal, *NA* bees-non Apidae; *Pl* Plecoptera, *T* thrips, *TR* Trichoptera; *W* wasp)

Plant Family and Species	Floral Form	MS	SS	Nectar	Beetle Families	Other* Vectors
Arecaceae						
<i>Asterogyne</i> (1)	B	+	+	+	Cur	D
<i>Socratea</i> (1)	B	+	?	?	?	NA
Asteraceae						
<i>Gorteria</i> (1)	PB	+	?	?	Scar	D
Balanophoraceae						
<i>Balanophora</i> (1)	B	+	+	+	Nit	D, L, M?
Caprifoliaceae						
<i>Viburnum</i> (1)	PB-B	+	?	+	Scar	D, NA
Flindersiaceae						
<i>Flindersia</i> (1)	B	+	+	?	Scar	D, L
Euphorbiaceae						
<i>Aporusa</i> (1)	B	+	?	-	Scar	D
<i>Baccaurea</i> (1)	B	+	?	-	Can, Scar	D
<i>Cephalomappa</i> (1)	B	+	?	-	Cur, Chry	D
<i>Drypetes</i> (2)	PB	+	?	+	Chry, Mord Scar	D
<i>Tapoides</i> (1)	PB-C	+	?	+	Ant, Elat Scar	D
Hyacinthaceae						
<i>Daubenya</i> (1)	PB	-	-	-	Scar	NA
<i>Ornithogalum</i> (1)	PB	-	-	tr	Scar	D
Illiciaceae						
<i>Illicium</i> (1)	C-PB AP	+	+	+	Can, Car, Ceram, Chry Curc, Elat, Lag, Scrap	D, NA Pl, TR
Iridaceae						
<i>Aristea</i> (1)	PB	-	-	-	Scar	AP
<i>Gladiolus</i> (1)	BL	-	-	+	Scar	NA
<i>Homeria</i> (2)	PB	-	-	-	Scar	D, AP
<i>Ixia</i> (1)	PB	-	-	tr	Scar	D
<i>Romulea</i> (1)	PB	-	-	?	Scar	NA
<i>Sparaxis</i> (3)	PB	-	-	tr	Scar	D
Liliaceae						
<i>Calochortus</i> (26)	PB	-	?	+	Bruch, Bup, Ceram, Cler, Mel, Mord	A, D, NA
Loranthaceae						
<i>Nuytsia</i> (1)	PB-B	-	?	+	Bup	W

Table 3 (continued)

Plant Family and Species	Floral Form	MS	SS	Nectar	Beetle Families	Other* Vectors
Mimosaceae						
<i>Acacia</i> (4)	B	+	+	-	Bup, Carab, Ceram, Cler, Scar	D, NA
Myrtaceae						
<i>Angophora</i> (1)	B	+	?	+	Canth, Ceram, Cler, Scar,	AP, NA
<i>Backea</i> (1)	PB	-	?	+	Bup, Can, Ceram, Mord, Scar	D, W
<i>Eucalyptus</i> (2)	B	+	?	+	Bup, Cler, Scar	NA, W
<i>Leptospermum</i> (1)	PB	+	?	+	Bup, Scar	NA
<i>Melaleuca</i> (1)	B	+	?	+	Bup, Scar	NA
<i>Verticordia</i> (1)	PB	+	+	+	Bup, Scar	NA
Nelumbonaceae						
<i>Nelumbo</i> (1)	PB-C	+	+	-	Can	AP, D, NA
Orchidaceae						
<i>Coeloglossum</i> (1)	BL	-	?	+	Can	D, W
<i>Epipactis</i> (1)	BL	-	+	+	Coc, Oed	D, AP, W
Paeoniaceae						
<i>Paeonia</i> (1)	PB	+	-	-	Scar	NA
Pittosporaceae						
<i>Bursaria</i> (1)	PB	+	?	+	Bup, Scar	D, L, W
Proteaceae						
<i>Leucospermum</i> (3)	B	+	+	+	Scar	AP, BI
<i>Protea</i> (15)	B-PB	+	+	+	Scar	BI
Rhamnaceae						
<i>Alphitonia</i> (10)	PB	-	+	+	Can, Chry, Coc, Cur, Derm, Lyc, Lag, Mord	D, NA, W
Saururaceae						
<i>Saururus</i> (1)	B	+	+	-	Scar	AC, AP
Trochodendraceae						
<i>Trochodendron</i> (1)	PB	+	?	+	Coc, Harp, Lag	AP, D, L
Winteraceae						
<i>Drimys</i> (1)	PB	+	+	-	Cur, Mord, Nit	D, W, T

again, several beetles may enter or forage on the same compound branch at the same time.

Polyandry is often considered a shared trait of beetle-pollinated systems (Gottsberger 1977, Bernhardt and Thien 1987) with large, bisexual flowers but this trait is absent in most petaloid monocots (e.g. *Tulipa*, *Orchidantha*, *Spiloxene* and all *Iridaceae*) due to genetic constraints. These flowers may compensate for the lack of

stamens by producing disproportionately large anthers (e.g. *Tulipa*; Dafni et al. 1990).

As the compound inflorescences of palms and aroids behave as if they were single flowers the multi-staminate condition is recapitulated in these largely tropical families of monocotyledons. It is also possible that massing together small, eudicot flowers, depauperate in stamens, may produce a multistaminate

condition in some inflorescences of beetle-pollinated members of the Polemoniaceae (*Ipomopsis*; Grant and Grant 1965) and Dipterocarpaceae (Momose et al. 1998). Regardless of reproductive unit most beetle-pollinated flowers are radially symmetrical (Table 2) excluding two, *Lowia* spp. (Sakai and Inoue 1999) and all orchid genera (Tables 2, 3; Singer and Cocucci 1997).

Much emphasis has been placed on the strong fragrances of flowers pollinated by beetles but odors differ broadly between species in different families. Odors have been described variously as fecal (*Lowia*; Sakai and Inoue 1999), musky (*Ipomopsis congesta*; Grant and Grant 1965), honeylike (*Homeria elegans* and *Gladiolus meliusculus*; Goldblatt et al. 1998), fruity (*Magnolia*; Dieringer et al. 1999) or reminiscent of alcoholic beverages and man-made food products (Nymphaeaceae; Prance and Arias 1975, Schneider and Moore 1980). The few scent analyses made on beetle-pollinated species indicate that both the number of scent notes and the concentration of compounds vary within a genus (Thien et al. 1975). However, floral scents are faint or undetected in a large proportion of beetle-pollinated herbs (Dafni et al. 1990, Goldblatt et al. 1998)

Beetle pollinators seek out flowers for three interconnected reasons. First, the majority of beetles forage for edible rewards in the flowers although the range of rewards varies considerably according to the mode of floral presentation (Table 2 and see below). Second, beetles often use the flower as a site to complete agonistic and mating activities (Gottsberger 1977, 1988, Goldblatt et al. 1998). In fact, beetle behavior may actually reflect differential rates of time male and female insects spend on the same flower (Dafni et al. 1990). Third, some beetles enter flowers because the blossom produces a physical temperature preferable to the external environment (Bay 1995, Seymour and Schultze-Motel 1997, 1998). Thermogenesis appears to be a character linked to mode of floral presentation (see below). This predictable behavior helps explain why both individual flowers and compound inflorescenc-

es pollinated by *Coleoptera* are often disproportionately larger than their pollinators. An increase in the physical size of the reproductive unit maximizes the number of beetles in or on a blossom encouraging interactive insect behaviors leading to pollen deposition on the vectors and contact between vectors and receptive stigmas.

As edible rewards are so variable within beetle-pollinated flowers (Table 2) it is not understood whether all beetle pollinators actively consume the pollen of their host flowers. Pollen collecting mouth-parts are discussed and illustrated by Barth (1985) and Bernhardt (1996) reviews the three different mechanisms beetles may employ to digest the contents of pollen grains. Peringuey (1902) also examined the mouth-parts of some flower-visiting genera in the Scarabaeidae and noted "suctorial" structures implying a diet that includes nectar.

The consistent presence of certain beetles on the flowers of certain plant families may merely reflect overlapping distributions instead of long-term interactions. Flowers pollinated by species in the family, Melyridae (Dasytidae s.s.), appear restricted to the western half of North America (Grant and Grant 1965, Dilley 1999). Woody plants pollinated, in part, by beetles in the family, Buprestidae, are most common in Australia (Hawkeswood 1987a, b, c).

Brush and bilabiate modes of presentation

Floral form subdivides into four modes of presentation in beetle-pollinated flowers although floral characters overlap (Tables 2, 4). In fact, two modes of presentation may be found on the same plant. Male flowers of *Tambourissa* spp. have Brush forms while the female flowers are Chamber Blossoms (Table 2; Lorence 1980).

Of the four modes, Brush and Bilabiate (gullet) flowers are least common. Brush blossoms usually consist of many, small, often unisexual flowers with reduced, or absent perianth segments massed together but devoid of overarching-enveloping bracts at the time

when anthers dehisce and stigmas are receptive. The inflorescence is held erect-nodding at anthesis. Beetles are exposed to view as they forage for pollen, stigmatic secretions or graze on floral organs. This mode of presentation is relegated to some palms (Schmid 1970, Essig 1971, 1973) an unidentified *Piper* sp. (Momose et al. 1998), *Ipomopsis congesta* (Grant and Grant 1965) and the aforementioned male flowers of *Tambourissa*. Palm and *Piper* inflorescences are usually elongated rods, drab-white flowered and variously scented-scentless. Some palms are thermogenic and much of the floral activity occurs before dawn (Essig 1971, 1973, Henderson 1984). With important exceptions, beetles with small bodies are the major pollinators of Brush inflorescences especially curculionids, nitidulids and staphylinids. Weevils in the genus, *Nodocnemus*, may occur exclusively in palm flowers (Essig 1973).

Variations in Brush form and presentation is best noted in *Ipomopsis congesta*. This species has rounded inflorescences that are polymorphic in color, musky-sweet scents and is pollinated primarily by small-bodied, melyrid beetles (Grant and Grant 1965).

The Bilabiate (gullet) mode of presentation is confined to the Lowiaceae and Orchidaceae (Table 2). This is the only beetle-pollinated mode expressing bilateral symmetry with one petal of the flower functioning as a broad landing platform or labellum (Singer and Cocucci 1997, Sakai and Inoue 1999). These bisexual flowers are usually held horizontally. They release and receive pollen or pollinaria employing a column or column-like organ. Pigmentation patterns on perianth segments may include dark spots or blotches against a lighter background similar to the "beetle marks" associated with Painted Bowl flowers (see below). These color patterns are not always consistent within the same Bilabiate species (Sakai and Inoue 1999). Floral odor also varies in tone and intensity.

Beetles do not appear to forage actively for pollen or pollinaria in Bilabiate flowers. *Pteroglossaspis ruwenzoriensis* is the only beetle-pollinated species known to offer nectar in a

gelatinized state (Singer and Cocucci 1997). The remaining species with Bilabiate flowers appear to be pollinated by deceit (Sakai and Inoue 1999, Steiner 1998b) or offer liquified nectar (Wallace 1980).

The chamber blossom

Reproductive units within this third mode of presentation have been described variously as chambered, urceolate, vase-shaped, haplo-morphic or forming a trap-like perigon (*sensu* Vogel 1978). The Chamber blossom is the most well studied and most frequently documented mode of beetle-pollination (Table 2). This includes members of the Araceae and Cyclanthaceae in which whole inflorescences are enclosed within one, or more, expansive bracts (Bay 1995, Beach 1982, Momose et al. 1998, Monteith 1973, Sivadasan and Sabu 1989, see review by Young 1986).

Most beetle-pollinated magnoliids are Chamber Blossoms (Endress 1990). In the Myristicaceae the chamber consists of the small but connate perianth (Armstrong 1986, Armstrong and Irvine 1989, Irvine and Armstrong 1988, Yeo 1993). In female flowers of *Tambourissa* spp. the chamber is composed of the thickened, floral receptacle (Lorenz 1980). The most common magnoliid chamber consists of a few to many inwardly cupped, perianth segments arranged in a continuous spiral or repetitive, overlapping whorls (e.g. Annonaceae, Calycanthaceae, Eupomatiaceae, Magnoliaceae etc.).

The majority of Chamber Blossoms are perfect flowers but some families (e.g. Araceae, Myristicaceae) or individual species (*Clusia criuva*) show a high proportion of imperfect flowers or florets. Chamber Blossoms with perfect flowers or compact inflorescences (consisting of male and female florets) show a far higher frequency of protogyny than the Bilabiate, Brush or Painted Bowl (see below) modes (Bernhardt and Thien 1987).

Chamber Blossoms are held erect to nodding at anthesis often accommodating dozens

of beetles within the floral interior (Thien 1974, 1980). The flower or inflorescence must produce a distinctive, “cave-like” interior sheltering and concealing beetles within a deeply inflated spathe (e.g. Araceae), or a connate tube (e.g. Myristica) or under a roof composed of overlapping bracts (e.g. Cyclanthaceae), petals (e.g. Annonaceae), or tepals (e.g. Magnoliaceae). The literature suggests that nocturnal activity is most extensive in this mode with opening flowers, scent production and the arrival of prospective pollinators discernible by dusk (Cramer et al. 1975, Prance and Arias 1975, Prance 1980, Thien 1980, Gottsberger 1989a, b) or after midnight (Rodrigues et al. 1993).

To the human eye pigmentation patterns appear insignificant, monotonous or inconsistent on bracts or perianth segments of most Chamber Blossoms. Most fieldworkers report pale colors (white, pink, cream-light yellow) although *Sphaerothalamus insinis* (Annonaceae) has red tepals (Momose et al. 1998). Irregular, dark purple-brown blotches appear more often on Chamber Blossoms than symmetrical nectar guides (Thien et al. 1974, Nelson and Ysaleny 1992, Momose et al. 1998). Some populations of *Linanthus parryae* are major exceptions as their flowers are blue with a stereotyped yellow annulus encircling the floral throat (Grant and Grant 1965). However this pattern is not consistent throughout the distribution of this polymorphic species. The presence of ultra violet patterns remains an under explored character in Chamber Blossoms but it is possible that such patterns exist in some *Magnolia* spp. contrasting tepals with floral receptacles and stigmas (Thien et al. 1995).

In contrast, floral fragrances are strong and very variable between species of Chamber Blossoms. The majority of species bear anthers that shed or extrude pollen (Bernhardt and Thien 1987, Bernhardt 1996) with secondary presentation of shed pollen restricted to the connate tube in *Myristica* (Yeo 1993).

Of all four modes of presentation the Chamber Blossom produces the most diverse

range of rewards. While comparatively few secrete floral nectar (Grant and Grant 1965, Norman and Clayton 1986) many beetles consume pollen (Table 2; Bernhardt and Thien 1987, Bernhardt 1996). A growing body of literature suggests that magnoliids offer edible rewards unique to systems pollinated by beetles and a few other insects (e.g. thrips and cockroaches) with short mouth-parts. These include reports of beetles consuming stigmatic secretions (Bernhardt and Thien 1987, Momose et al. 1999), food bodies (Grant 1950) and specialized floral organs (Table 2). For example, in *Eupomatia laurina* both adult weevils and their larvae eat staminodia within the synandrium (Armstrong and Irvine 1990). The floral epidermis of *Exospermum stipitatum* contains microscopic, polysaccharide food-bodies consumed by weevils (Thien et al. 1990) but visibly large, starchy bodies form a barrier between stamens and carpels in *Victoria* (Prance and Arias 1975). It is unclear whether some Chamber Blossoms offer edible rewards throughout the floral life span. Beetles that do not consume stigmatic secretions (protonectar) may have no other edible rewards during the female phase of some protogynous Chamber Blossoms or inflorescences. This implies automimicry in some aroids, *Magnolia praecocissima* var. *borealis* (Ishida 1996) and in all female flowers of *Clusia criuva* (Rodrigues et al. 1993) and *Tambourissa* (Lorenz 1982).

Thermogenicity is more common in Chamber Blossoms compared to the remaining three modes of beetle-pollination (Gottsberger 1989a, Seymour and Schultze-Motel 1997, Dieringer et al. 1999). While heat production is not detected in all Chamber Blossoms it appears to have evolved independently in both magnoliid and monocot families.

Considering the wide variety of floral scents and edible rewards it is not surprising that Chamber Blossoms attract the greatest diversity of beetle pollinators. The majority of publications report pollination by beetles in the families Curculionidae, Nitidulidae, Scarabaeidae and Staphylinidae. However, com-

pared to the remaining three modes of floral presentation Chamber Blossoms also receive the greatest number of visits by beetles in the families Anthicidae, Cerambycidae, Chrysomelidae, Elateridae, Hydrophilidae, Mordellidae and Scraptiidae (Table 2).

Chamber Blossoms should not be regarded as primitive modes of floral presentation exclusive to magnoliids and basal monocotyledons. This mode of presentation has evolved independently in much derived families of eudicots including the Polemoniaceae (Grant and Grant 1965), Clusiaceae (Rodrigues et al. 1993), Convolvulaceae, Dipterocarpaceae and Sterculiaceae (Momose et al. 1998, Sakai et al. 1999). Chamber Blossoms manufactured by species in these families duplicate almost all the floral characters discussed above except that genetic constraints may prevent them from duplicating the multi-staminate androecia and the multi-whorled and multi-segmented perianths found in magnoliids bearing large flowers.

The pollination ecology of some Chamber Blossoms reflects varying levels of specialization within members of the same lineage. For example, within the Magnoliaceae, *Magnolia* spp. native to temperate, southeastern regions of North America appear to be generalists pollinated by beetles representing six to eight families (Thien 1974, Piegler 1988). In contrast, some neotropical species are more specialized. Two *Magnolia* spp. of montane, Mexico are pollinated primarily by *Myrmecoccephalus* sp. (Staphylinidae) and *Cyclocephala jalapensis* (Scarabaeidae; Dieringer et al. 1999). *Talauma ovata* is pollinated primarily by *Augoderia* spp. (Gibbs 1977).

Intra and intergeneric variation of beetle pollinators is well documented within the Annonaceae (Gottsberger 1977, 1988, 1989a, b). In *Annona* s.s. Gottsberger (1989a, b) discriminates between species pollinated primarily by small-bodied nitidulid and staphylinid beetles vs. those pollinated almost exclusively by large-bodied scarabaeids.

Some Chamber Blossoms are pollinated by a single genus or species of beetle (Grant 1950,

Grant and Grant 1965, Sivadasan and Sabu 1989, Armstrong and Irvine 1990). Pollination by a solitary genus of scarabaeid is particularly common (Monteith 1973, Cramer et al. 1975, Prance and Arias 1975, Prance 1980, Beach 1982, Young 1986, Gottsberger 1989b). Beetles of several families visit male flowers of *Clusia criuva* but *Dinaltica bahiaensis* (Chrysomelidae) is the only insect that visits both male and female flowers (Rodrigues et al. 1993). Similar observations made on so many unrelated plant species can no longer be dismissed as bias based on inadequate insect collections.

Painted bowls

The final mode of beetle-pollination is described as the Painted Bowl. It is a highly localized system reaching its greatest diversity in regions experiencing Mediterranean climates in southern Africa and the southeastern Mediterranean basin (Table 1). Most Painted Bowls are bisexual flowers held erect-horizontal at anthesis. The perianth forms a deep cup-flattened salver but rarely consists of more than two whorls of segments. Perianth segment number is usually fixed and rarely consists of more than a total of six petals or tepals in petaloid monocotyledons and five petals in most advanced eudicotyledons (Goldblatt et al. 1998). Therefore, both beetles and the sexual organs of the flower are not concealed inside a pouched bract or under a roof of perianth segments at anthesis.

Androecia of Painted Bowls vary from multi-staminate and multi-whorled (e.g. *Ranunculus asiaticus*, *Papaver rhoes*) to single-whorled with only three stamens/flower (e.g. Iridaceae). Flowers with few stamens often bear comparatively large anther sacs that shed pollen (e.g. *Tulipa agenensis*; Dafni et al. 1990) but this character is far more common in Chamber Blossoms (see above). In the beetle-pollinated Iridaceae of southern Africa anthers may be aligned with elongated stigma lobes forming a staminal column that swabs pollinators as they forage or copulate within the perianth bowl (Goldblatt et al. 1998, Steiner

1998a). It is noted here, though, that columns are not unique to beetle-pollinated irids and modification of column morphology may parallel the physical size, taxonomic range and foraging behavior of major pollen vectors (Goldblatt and Bernhardt 1999).

Floral attractants expressed by the Painted Bowl are the inverse of Chamber Blossoms. Painted Bowls do not bloom at night. Few fieldworkers note floral fragrances. Sweet scents are uncommon and noxious odors are almost nonexistent (Table 2). Thermogenesis remains unrecorded in these flowers. Instead, Painted Bowls are far more likely to show vivid but regular patterns of pigmentation. While Picker and Midgley (1996) emphasized blue-violet and white in South African species pollinated by two beetle genera the full range of colors is far more variable. Red-orange dominates Painted Bowls of the southeastern Mediterranean from late winter–mid spring (Dafni et al. 1990). Yellow-orange and various shades of pink are common in the Painted Bowls of South Africa (Goldblatt et al. 1998) but ultra violet patterns remain uninvestigated.

Regardless of plant family the petals of Painted Bowls often bear “beetle marks” (Table 2). Beetle marks on petals or sepals are symmetrically oriented, stylized spots that are usually brownish-black to dark iridescent (Steiner 1998a) in tone and confined to the base of the perianth. A beetle mark is often outlined with a curved, contrasting band or fleck of white or some lighter color (Goldblatt et al. 1998). Some of the herbs of the Mediterranean basin extend the darkened, floral center by blackening their anthers or whole stamens (e.g. *Anemone*, *Papaver*, *Tulipa*; Dafni et al. 1990).

The function of beetle marks is unclear. Most Painted Bowls fail to secrete nectar so the beetle mark is not a nectar guide. However, field experiments by Dafni et al. (1990) on flower pollinating scarabs showed that *Amphi-coma* spp. are significantly more attracted to red model flowers containing a central, darkened spot than to a pure red model. Scarab

beetles of South Africa continue to visit artificial flowers in which beetle marks are presented asymmetrically (Midgley and Johnson 1998).

As Painted Bowls remain untested for heat production or absorption the temperature of the beetle mark should be compared to both ambient temperatures and the more lightly colored parts of the flower. This would determine if the beetle mark absorbs more heat on a sunny day. Phenological records show clearly that many Painted Bowls bloom during cool seasons, from late winter through early spring (Dafni et al. 1990, Goldblatt et al. 1998). If beetles seek out warm spots on the Painted Bowl, as they do in many Chamber Blossoms, then the beetle mark would help position the pollinator next to, or on top of, the stamens or staminal column.

It is also noted that pigmented pollens contrasting with the outer, perianth lobes are common in Painted Bowls. Note that *Tulipa* and *Papaver* spp. of the southeastern Mediterranean offer grains with blackish pollen coats (Dafni et al. 1990). Goldblatt et al. (1998) found that 0.32 of 43 species of Painted Bowl flowers (half of the 16 genera studied) offered either bright red-orange or blue-brownish black pollen.

Pollen is the only consistent edible reward offered by the Painted Bowl. Nectar secretion is recorded in this beetle-pollinated mode but secretions are often recorded as trace amounts (Goldblatt et al. 1998). Food bodies and edible staminodia remain unrecorded in this mode. This helps explain the lack of reports of protogyny and automimicry in these flowers compared to Chamber Blossoms.

Floral presentation emphasizing open perianths, visual instead of olfactory cues and rewards limited largely to pollen also help to explain the limited diversity of beetle pollinators in Painted Bowls. The majority of Painted Bowl pollinators belong to genera in the Scarabaeidae with hairy bodies (Table 2). While the range of pollinator diversity is much more limited in Painted Bowls than in Chamber Blossoms the degree of coadaptation

between Painted Bowls and scarabaeids remains variable.

Steiner (1998a) associated certain species of scarabaeids (tribe *Hopliini*) with the pollination of certain *Moraea* spp. Dafni et al. (1990) noting temporal successions between six species of scarab pollinators (*Amphicoma* spp.) and the overlapping phenologies of four, herb species with red-orange flowers. Peak emergence of *A. aleppensis* and *A. libanonensis* coincided much more closely with peak flowering of *Ranunculus asiaticus* and *Tulipa agenensis* than with *Papaver rhoeas*. As populations of *A. genei* do not peak until after mid-late April it is more important as a pollinator of *P. rhoeas* than as a pollinator of the *Anemone* and *Tulipa* spp. of February–March (Dafni et al. 1990).

In contrast Goldblatt et al. (1998) collected 26 species of beetles, representing nine, scarab genera on the flowers of 43 species of herbaceous plants representing four families native to southern Africa. Insect and plant identifications cross-referenced with pollen load analyses failed to provide sufficient evidence favoring any beetle species as a specific forager and pollinator on any plant species. Insect collections and pollen load analyses of scarab beetles in southern Africa suggest that Painted Bowl flowers depend on more generalist foragers (Picker and Midgely 1996) in the *Hoplinii*. A single, adult insect, regardless of genus, visits different species of coblooming Painted Bowls bearing the pollen of two-five different plants prior to capture (Goldblatt et al. 1998).

The role of beetles in generalist pollination syndromes

It is understood that beetles contribute to the pollination of species that exploit other flower visiting insects and vertebrates (Table 3). Unfortunately, few studies compare the efficiency of beetles as pollen vectors vs. other animals visiting the same flower. For example only 6% of the pollinaria produced by flowers of *Epipactis palustris* (Orchidaceae) are removed

by beetles as the vast majority of flower visitors are nectar-drinking wasps (Nilsson 1978).

In contrast *Cetonia* spp. (Scarabaeidae) prefer flowers of *Viburnum opulus* to other coblooming species. While insects in other Orders pollinate flowers of *V. opulus* Englund (1993) found that *Cetonia* spp. may be the most dependable long-distance pollinators flying an average of 18m between plants with 26% of marked beetles returning annually to the same shrubs.

As discussed above there is an obvious overlap between families of *Coleoptera* in specialist (Table 2) vs. generalist (Table 3) flowers. Specifically taxa belonging to the beetle families Cerambycidae, Curculionidae, and Scarabaeidae are most likely to pollinate both specialist and generalist systems (Tables 2, 3). Further overlap occurs in the less common families of Cleridae and Mordellidae in generalist and specialist blossoms. Does this mean that the characters associated with flowers pollinated primarily by beetles have been overemphasized? It would be easy to dismiss specialized, beetle-pollinated flowers as the overzealous dogma of evolutionary botanists supporting an adaptationist agenda. A current school of thought argues that the emphasis of rigidly specialized syndromes is counterproductive to field study as generalist pollination is clearly the rule, not the exception, in floral evolution (Waser et al. 1996).

Based on interpretations of Tables 1–3 the evolution of beetle-pollination represents a real gradation from generalist to specialist systems. Specialized systems are identified so frequently in certain habitats (Table 1) it is unlikely they are all misinterpretations based on strict adherence to dogma. As specialized, “beetle flowers” actually consist of four, overlapping modes of floral presentation it is predictable that certain characters are also found in species with more generalist syndromes. In particular, if we compare the characters of Chamber Blossoms and Painted Bowls against generalist systems known to incorporate beetles into the vector spectrum few characters appear exclusive to specialized beetle-pollination (Table 4).

Table 4. Comparative Characters of Three Reproductive Modes Pollinated Primarily By Beetles vs. Those Pollinated Partly By Beetles. + = character present and dominant (occurs in 0.50 or >0.50 of taxa examined); + - character present but not dominant (occurs in <0.50 of taxa examined); - = character absent

Reproductive Characters	Chamber Blossom	Painted Bowl	Generalist Systems Incorporating Beetles and other animals
Nocturnal Flowering	+ -	-	-
Primary Attractants			
Beetle Marks	-	+	+ -
Strong Scents (fecal, nutty, fruity, musky)	+	+ -	+ -
Thermogenesis	+	?	+ -
Bracts			
Inflated-Enveloping	+	-	-
Petaloid and Colorful	-	-	+ -
Perianth			
Multi-segmented	+	+ -	+ -
Multi-whorled or Replaced by spathe	+	-	+ -
Androecium			
Nocturnal dehiscence	+	-	-
Multi-staminate	+	+ -	+ -
Extrusive anthers	+	+ -	+ -
Stamens or anthers			
Black contrasting with perianth	-	+ -	+ -
Pollen coat colorful contrasting with floral organs	-	+	+ -
Edible Rewards			
Food Bodies and other edible tissue	+	-	-
Stigmatic Secretions (protonectar)	+	-	+ -
Whole organs (staminodia, synandria, perianth segments)	+	-	-
Pollen	+	+	+
Nectar	+ -	+ -	+

While Chamber Blossoms attract the greatest diversity of beetles they appear to represent a more specialized mode of pollination than the Painted Bowl. Generalist systems that incorporate beetles and other animals do not appear to employ nocturnal anthesis in conjunction with specialized food bodies and inflated, chamberlike bracts or perianths. Note also that generalist systems are more likely to employ complex color patterns absent in most Chamber Blossoms (Table 4). Magnoliids (e.g.

Drimys brasiliensis and *Illicium floridanum*) pollinated by insects representing several Orders have flattened, salverform, perianths consisting of many tepals that fail to form the standard, chambered roof (Gottsberger et al. 1980, Thien et al. 1983).

Nelumbo flowers are often interpreted as classic examples of beetle-pollination but field-work in North America confirms that *N. lutea* (syn. *N. pentapetala*) is pollinated by a wide range of insects including beetles, bees and flies

(Sohmer and Sefton 1978, Schneider and Buchanan 1980). While beetles in the genus, *Chauliognathus* (Cantharidae) are considered dominant pollinators of *N. lutea* at some sites observations in the American midwest show they do not always persist throughout the flowering period and are poor pollen vectors. Female bees that collect pollen and drink stigmatic secretions appear essential for pollination of this species (B. J. Bullins and P. Bernhardt, personal observations). There is no doubt that *N. lutea* shows most of the characters of a Chamber Blossom including protogyny, strong scent, thermogenesis, polyandry and extrusive anthers. However the development of its internal chamber has been suppressed thoroughly by the elongation and expansion of the fleshy receptacle forming the flattened, carpophore disc. The surface of the disc is almost parallel with the petal tips the first day the corolla opens. The disc elevates the receptive stigmas turning the flower chamber into a flattened tray for the duration of the floral life span.

Large blossoms pollinated by a combination of large-bodied beetles and other animals have also evolved in the *Proteaceae* via modification of compact synflorescences and their subtending bracts. In South Africa some *Protea* spp. may be pollinated by scarabs, other insects and vertebrates (Collins and Rebelo 1987). While *Protea* florets form dense, nectar-secreting brushes on the massive peduncle the base of the synflorescence is usually encircled by many, colorful, petaloid bracts forming a compound version of the Painted Bowl.

Therefore, it must be emphasized that no trait employed by Chamber Blossoms and Painted Bowls, by itself, is unique to systems pollinated exclusively by beetles (Table 4). The concept of specialized vs. generalist pollination syndromes remains viable provided each syndrome is regarded as an interrelated suite of characters with variation possible at each character state. As mentioned above, thermogenesis and strong odors occur in the generalist, *Nelumbo lutea*. They also occur in many aroids pollinated primarily by true flies (Sey-

mour and Schultze-Motel 1997, 1998) and are suspected in generalist *Illicium* (Table 3; L. B. Thien, personal communication). Black marks on petals occur commonly in the Asteraceae of southern Africa when flowers are pollinated by a combination of beetles and other insects (Johnson and Midgely 1997, Goldblatt et al. 1998, Midgely and Johnson 1998). Protogyny is common throughout magnoliids and other relic angiosperms but occurs independently of beetle-pollination (Bernhardt and Thien 1987). Beetle-pollination, like most syndromes, can not be predicted on the basis of one or two "signature traits."

In fact, character suites encouraging beetle-pollination in generalist systems may be so subtle that they typify different Sections within the genus. Pollination of *Calochortus* spp. emphasizes both beetles and bees. However, beetles are more common as pollinators in Section *Mariposa* than in Section *Calochortus* (Dilley 1999).

Furthermore, character suites associated with beetle-pollination do not ensure pollination by beetles when plant distribution changes. *Nuphar lutea* is a Chamber Blossom native to North America (Table 2, Schneider and Moore 1977) but naturalized populations in northern Europe are more likely to be pollinated by social bees and syrphid flies than by the chrysomelid beetle, *Donacia crassipes* (Renner and Johanson 1995).

The literature suggests that the Australian flora is unusually rich in generalist pollination systems combining beetles with other insects (Armstrong 1979, Irvine and Armstrong 1988). The Chamber Blossom of *Eupomatia laurina* is one of the few, Australian endemics pollinated exclusively by beetles (Irvine and Armstrong 1988, Armstrong and Irvine 1990). In contrast, generalist pollination by beetles and other insects extend from Australian rainforest to Mediterranean shrublands and temperate woodlands appearing in such unrelated families as Loranthaceae, Myrtaceae and Pittosporaceae (Hawkeswood 1980, 1981a, b, 1982, 1987a, b, c, 1989, 1990, 1992, 1993). Some of these exhaustive contributions by Hawkes-

wood may give the false impression that there are Australian species of Myrtaceae pollinated exclusively by beetles but this is the result of unfamiliarity with the author's research program. Hawkeswood was most interested in the role of beetles as pollinators and often declined to collect other floral visitors. Brush (e.g. *Angophora*, *Eucalyptus* and *Melaleuca*) and bowl-shaped (e.g. *Baeckea*, *Leptospermum* and *Verticordia*) flowers dominate the Myrtaceae pre-adapting them for visits by beetles and other insects with short mouth-parts. In fact, Australia is the center of diversity for the short-tongued bee family, Colletidae (Michener 1979). Collections, observations and pollen load analyses by other authorities indicate that most animal-pollinated Myrtaceae are generalist flowers combining beetle pollinators with a broad range of flies, native bees and nectar feeding birds (Armstrong 1979, Bernhardt 1989, Bernhardt and Weston 1996). This is not to assume that specialized beetle-pollinated systems do not exist in Australia outside rainforest remnants. The role of beetles as primary pollinators remains to be assessed in the Myrtaceae (the most diverse family in Australia) especially in taxa in which the central nectary covering the floral receptacle is a deep iodine-maroon in color resembling a beetle mark (Bernhardt, personal observation).

Generalist systems pollinated by beetles and other animals appear more likely to offer nectar as a reward than Chamber Blossoms or Painted Bowls (Tables 2) although several generalist species secrete only trace amounts of fluid (Table 3). As many pollinators are unable to digest pollen or consume starchy food bodies (Bernhardt 1996) nectar secretion encourages additional floral foragers without discouraging beetles. Chaw (1992) notes that beetles consume the pollen of *Trochodendron aralioides* ignoring nectar consumed by other insect visitors.

A number of flowers fail to secrete nectar yet continue to mix beetles with other pollinators (Table 3). These include some petaloid monocots of southern Africa (Goldblatt et al. 1998), Australian *Acacia* spp. (Bernhardt 1989), *Paeonia* (Yi-Bo et al. 1999) and *Sauru-*

rus (Thien et al. 1994). The absence of nectar appears to limit the diversity of floral foragers emphasizing beetles that share flowers with pollen-eating syrphid flies and with polylectic bees foraging for pollen to provision offspring. This means that these generalist systems are really closer to the Papaver-type of pollen flower as first reviewed by Vogel (see reinterpretation by Bernhardt 1996).

This is not to suggest, though, that beetles refuse to feed on nectar. Beetles are well documented consuming protonectar secreted by stigmatic surfaces in Chamber Blossoms (Thien et al. 1995, Bernhardt 1996, Momose et al. 1998). More important, some genera of Australian buprestids and scarabaeids have mouthparts modified for the collection of liquids. These beetles are observed and photographed pushing through, or climbing over dehiscent stamens to feed on nectar that accumulates around the gynoecium (Hawkeswood 1980, 1981b, 1987b, c, 1989, 1990). Dilley (1999) recorded 11 genera of beetles feeding on the nectar glands of *Calochortus* spp.

Distribution of the beetle-pollinated flora

As discussed above, beetle-pollination occurs more commonly in tropical-Mediterranean regions but this requires some qualification (Table 1). When Mediterranean biomes are compared it appears that the Painted Bowl mode is expressed by only a fraction of the species distributed through the eastern Mediterranean basin even though these few, scarab-pollinated herbs often form dense, local populations (Dafni and O'Toole 1994). In contrast, over a third of all herbaceous species native to the sand plain fynbos of South Africa are scarab-pollinated (Picker and Midgely 1996). Furthermore, dipterocarp forests of the Paleotropics may contain a far higher proportion of beetle-pollinated species (Momose et al. 1998) than lowland rainforests of Mesoamerica (Bawa et al. 1985). If the Appendix in Momose et al. (1998) is accepted literally than the Indo-Malaysian forests are unusually rich in woody families (e.g. Celast-

raceae, Clusiaceae, Fagaceae, Flacourtiaceae, Lauraceae, Leguminoseae *s.l.*, Menispermaceae etc.) pollinated by a combination of beetles and other insects, especially flies.

The proportions of beetle-pollinated species may diverge within the same land-mass diverge according to altitude, plant phylogeny and life form. Over 45% of the monocotyledons in a South American cloud forest are beetle-pollinated (Seres and Ramirez 1995) independent of life form, compared to less than 3% of the herb flora in a palm swamp at a lower altitude (Nelson and Ysaleny 1998). None of the canopy trees in lowland rainforest of Mesoamerica are beetle-pollinated but >10% of sub-canopy trees are (Bawa et al. 1985).

The role of beetles as pollinators changes with disjunctive plant geography. Biodiversity of the *Proteaceae* is highest in Australia and southern Africa (Johnson and Briggs 1975). While scarabs are implicated as co-pollinators of some shrubby species endemic to southern Africa beetle-pollination is largely undocumented in the *Proteaceae* of Australia (Armstrong 1979, Collins and Rebelo 1985). The Western Hemisphere appears to be the center for beetle-pollinated *Nymphaeaceae* (Cramer et al. 1975, Prance and Arias 1975, Schneider and Moore 1977, Prance 1980) while Australia, as discussed above, has the greatest number of examples of generalist *Myrtaceae* with beetle pollinators. Of course, these generalizations may reflect sampling biases based on unbalanced histories of field studies of pollination mechanisms. In other plant families (e.g. Annonaceae and Magnoliaceae) beetles dominate pollination systems regardless of geography (Thien 1974; Thien et al. 1995; Gibbs 1977; Gottsberger 1988, 1989a, b; Momose et al. 1998).

Origins of beetle-pollinated floras

Takhtajan (1991) offers a list of authors who have supported the theory of Diels (1916) that beetles were the earliest pollinators of flowers. This theory is so pervasive it is still offered in

botany textbooks for undergraduate classes (see Moore et al. 1995, Northington and Schneider 1996) and has been addressed in technical and semi-technical publications for more than 80 years. Should the life of this theory be extended through the next century?

Bernhardt and Thien (1987) reviewed the literature on the pollination of primitive angiosperms and came to the conclusion that, while, beetle-pollination is very common in relic angiosperms it is too specialized a syndrome to apply exclusively to either the first angiosperms or their protoangiosperm ancestors. Independent research following the publication of Bernhardt and Thien (1987) continues to support the theory of an early but derived origin for specialized beetle-pollination in flowering plants. There is little doubt that specialization for beetle-pollination evolved in flowers by the mid-late Cretaceous as reconstruction of fossil flowers indicates that bowl and urn-shaped blossoms appeared on woody, reproductive stems 90–100 million years ago (Friis and Crepet 1987). However generalist, small-flowered, usually unisexual, Brush-type inflorescences (Koonwara-type; Platanoid-type) are 20–25 million years older than the bisexual, beetle flower (Friis and Crepet 1987). Both Chamber Blossoms and Painted Bowls could not have evolved until the later development of broad perianth segments and petal-like, enveloping bracts.

Dogmas arguing in favor of the ancestry of beetle-pollination require reconsideration in the light of recent evidence. It was once presumed, for example, that flower-visiting beetles belong to the oldest Order of insects associated with seed plants. Therefore, pollination by beetles had to predate pollination by insects in more recent Orders. This argument should be retired. The fossil evidence currently indicates that Orders containing at least one lineage of flower-visiting insect appeared before the earliest flowering plants (Gottsberger 1988, Labandeira 1998b, Grimaldi 1999). Extinct members of these Orders could have fed on gymnosperm pollen and/or ovule secretions 163–144 million years ago. Brachycera flies are

now dated to the late Jurassic (Ren 1998) while a fossil bee's nest, attributed to ancestors of the Halictidae, is dated to 220 million years (see Bernhardt 1999). The now extinct hypeperlid insects also appeared by the early Permian. Examination of fossils of their gut impressions and coproliths show that some of these insects ate pollen (Labandeira 1998a).

A second argument emphasizes beetle pollination due to beetle-pollination in allied gymnosperms. Bract and strobilus arrangements of fossil *Benettiales* are interpreted as Chamber Blossom prototypes (Crapet and Friis 1987, Gottsberger 1988) and some extant cycads are weevil (Curculionidae) pollinated (Norstog 1987). Based on fossil and living evidence there is little doubt that insect-pollination evolved first in gymnosperms. Does this mean, though, that beetle-pollination in seed plants predates all other entomophilous syndromes? Undoubtedly, insect-pollination systems in gymnosperms became specialized by the latter half of the Jurassic. However, insisting that beetle-pollination is older than any other pollination mechanism in the gymnosperm-angiosperm lineage ignores moth-pollination in *Gnetum* (Kato and Inoue 1994, Momose et al. 1999) and fly-pollination in both *Ephedera* (Bino et al. 1984) and cycads like *Zamia pumila* (Breckon and Ortiz 1983). Regardless of major vector the ovules of some entomophilous gymnosperms secrete a fluid that may also function as an edible reward comparable to the protonectar and true nectar of angiosperms.

The final argument is the oldest. It insists that beetle-pollination of angiosperms must be an archaic character because it is so well represented in basal families. Recent molecular based phylogenies can be used to support the theory. The review of Bernhardt and Thien (1987) took a pre-molecular approach using the standard taxonomy of Cronquist (1981). It is acknowledged here that molecular phylogenies, if they are correct, remove some bee-pollinated families (e.g. *Dilleniaceae*) from the original analyses of Bernhardt and Thien

placing a far greater emphasis on beetle-pollination within basal families.

However, molecular data does not change the interpretation of beetle-pollination derived from more generalist systems. Molecular treatments suggest that the monocotyledon clade may nest within basal magnoliids upholding the treatment of Arecaceae and Araceae as primitive families. This introduces more bee, fly and generalist pollination systems to a survey of the primitive angiosperms.

Concentration on pollination mechanisms within magnoliids still fails to make a convincing case for beetles as the first angiosperm pollinators because generalist systems are expressed within some Illiciaceae, Myristicaceae and Winteraceae while beetle, bee and fly-pollination subdivides other families (e.g. Lauraceae, Nymphaeaceae; Table 3; Bernhardt and Thien 1987). Cockroach and thrip pollination has been detected in some Annonaceae (Nagamitsu and Inoue 1997, Momose et al. 1998) and Chloranthaceae (Yi-Bo and Zhen-Yu 1999). Of far greater importance, when the functional, floral morphology of *Saururus* (Saururaceae) is compared to angiosperm fossils of the early Cretaceous (Thien et al. 1994) it argues more for the ancestry of generalist pollination incorporating air currents and mixed insects including beetles.

Of course, beetle-pollination must be regarded as one of the earliest modes of floral specialization. There can be no argument that gymnosperms bearing strobila suggestive of beetle-pollination predate all angiosperms (Crapet and Friis 1987) but it is just as likely that beetle, moth and fly pollination all evolved in angiosperms at much the same time to exploit guilds established by pre-existing, insect-pollinated gymnosperms. Beetle-pollination was derived, most probably, from generalist entomophilous ancestors based on the "weedy" habit of the first angiosperms (Gottberger 1988) and the comparatively late appearance of petaloid bracts and large perianth segments in the fossil record.

Floral parts are serially homologous organs. Historically serially homologous organs

are often modified following the interplay of Drift and Selection (Futuyma 1998). Therefore, the appearance of specialized beetle, or fly or bee-pollinated systems during the second half of the Cretaceous is based most probably on two interlocking features. First, labile sexuality is ancestral to the angiosperms. This encouraged modification of stamen and carpel number, size and functional morphology (see Endress 1990) in true flowers during the early Cretaceous. Second, convergent evolution in angiosperms encouraged their exploitation of specialized insect-pollinated guilds that had already been established in gymnosperms during the Jurassic.

What then is the oldest mode of floral presentation in beetle-pollinated flowers? The reconstruction of the flower of *Archeanthus linnenbergeri* (Dilcher and Crane 1984) argues that the Chamber Blossom predates all other modes pollinated primarily by beetles as the specimen is derived from an extinct magnoliid with a floral morphology paralleling beetle-pollinated members of the Magnoliaceae and Annonaceae.

It must be acknowledged that the proposed primacy of Chamber Blossoms is biased due to the limits of interpreting fossil specimens. As amber and sediment casts can't preserve original pigmentation it is impossible to pinpoint the appearance of the first Painted Bowls in the absence of "tell-tale" beetle marks. It should also be noted that fossils of some bowl-shaped, bisexual flowers with solitary whorls of petals or tepals are as old as *Archeanthus* (Friis and Crepet 1987). Interpretations of floral morphology ally these bowl flowers with families of some modern, woody eudicots including the *Myrtaceae*. It is possible that fossils of bowl-shaped flowers lacking impressions of nectar glands (Friis and Crepet 1987) could have belonged to woody angiosperms bearing Painted Bowls.

It is equally unclear as to which beetle lineage produced the earliest vectors of angiosperm pollen. Insect fossils and modern gymnosperm pollinators suggest early interactions between Nitidulidae (Crepet and Friis 1987),

Curculionidae and Jurassic ancestors of flowering plants. Pollen consumption may be an ancestral trait in the evolution of the Chrysomelidae and other beetle families (Samuelson 1994). While fossil evidence shows that flowering plants and representatives of modern beetle families coexisted during the Cretaceous how can we insist that a fossil of a phytophagous beetle must have belonged to a pollinator instead of a floral vandal or a fruit, ovule or seed predator?

Based on fossil reconstruction of flowers it's unlikely that specialized beetle-pollination in angiosperms appeared prior to the mid-late Cretaceous as earlier flower fossils suggest a combination of generalist entomophily (small insects with short mouth-parts) combined with pollen dispersal by air currents. Considering the relative ages of fossils representing extinct *Hymenoptera* and *Diptera* with the reconstruction of fossils of eudicot flowers (Crepet and Friis 1987, Friis and Crepet 1987) we can not insist that beetle-pollinated flowers predated specialized syndromes dependent on bees or flies.

Molecular treatments continue to emphasize the basal position of magnoliids within the angiosperms. Examination of late Cretaceous fossils of magnoliid flowers show floral organs and architecture consistent with some living, beetle-pollinated magnoliids. This includes many laminate, pollen-extruding stamens, multi-whorled perianths and carpels lacking true styles. Fossils of magnoliid flowers are so similar to extant taxa it permits us to propose that Cretaceous magnoliids with bisexual flowers and tepals may have also been protogynous, strongly fragrant, short lived (24–48 hours), thermogenic and capable of secreting edible, stigmatic exudate. However, as we have seen above, while all these morphological and physiological characters are common to magnoliid flowers they are not exclusive to beetle-pollination (Bernhardt and Thien 1987).

Beetle-pollination radiated beyond the magnoliid lineage. It evolved independently within 14 families of advanced eudicotyledons (Table 2) emphasizing the convergent origins

of both Chamber Blossoms and Painted Bowls. As the floral ecology of Mediterranean and rainforest biomes are surveyed the diversity of beetle-pollinated eudicots can only increase.

Beetle-pollination also evolved within the monocotyledons but the syndrome differs within the lineage. Three basal families (Arecaceae, Arecaceae, and Cyathophylacaceae) share some morphological (e.g. food bodies, stamens without filaments, carpels with short styles, labile sexuality), phenological (e.g. nocturnal flowering, protogyny) and physiological characters (e.g. strong scents, thermogenesis) convergent with cantharophilous magnoliids. However, these three families produce Chamber and Brush Blossoms based on compound inflorescences instead of individual flowers. In contrast beetle-pollination evolved independently in six families with petaloid flowers and herbaceous habits (Hyacinthaceae, Hypoxidaceae, Iridaceae, Liliaceae, Lowiaceae and Orchidaceae). These species produce both Painted Bowls and Bilabiate flowers based on individual flowers. It is also predicted that the diversity of beetle-pollinated monocotyledons will increase with surveys of moist tropical and Mediterranean biomes.

The adaptive radiation of beetle-pollination is based on two interactive factors. Specifically, phytogeography works in conjunction with convergent evolution. Both magnoliids and primitive monocotyledons are most diverse through tropical and subtropical habitats. These plants show a broad range of life habits within the moist, tropical biome (sub-canopy trees, herbaceous-woody vines, lithophytic-epiphytic herbs) and exploit beetle pollinators representing different families of *Coleoptera*.

Therefore, the long-term distribution and stability of guilds of beetle-pollinated magnoliids and basal monocotyledons in the tropics may have encouraged the convergent evolution of beetle-pollination within the more diverse lineage of advanced eudicotyledons (Tables 1, 2). While beetle-pollination within the wet tropics is as old as the mid-late Cretaceous the exploitation of beetle pollina-

tors by members of the Bombacaceae, Clusiaceae, Ebenaceae, Sterculiaceae etc. is probably recent and recurrent.

In contrast most Painted Bowl systems common through southern Africa and the eastern shores of the Mediterranean Basin appear to lack either a close genetic heritage with magnoliids and primitive monocotyledons or share habitats with an older, beetle-pollinated flora. Instead there is a recurrent and stereotyped trend towards beetle-pollination in both bulbous, petaloid monocotyledons and shrubby or rhizomatous-tuberous eudicotyledons (Tables 1, 2). Therefore the Painted Bowl systems of Mediterranean Eurasia and southern Africa are less variable than beetle-pollinated systems throughout the wet tropics because Painted Bowls must depend, almost exclusively, on hairy genera in the Scarabaeidae.

The canalization of beetle-pollination in southern Africa is only a small part of a multifaceted trend in the adaptive radiation of animal-pollinated systems incorporating rodents, birds, long-tongued flies, large butterflies, flower wasps (Masaridae) and bees with much modified proboscis or forelegs (Vogel 1954, Whitehead et al. 1987). Pollination by hairy scarabs converges within three families that have centers of species diversity within southern Africa; Iridaceae (Goldblatt and Bernhardt 1999, Bernhardt and Goldblatt, in preparation), Orchidaceae (e.g. *Disa*, Johnson et al. 1998) and Proteaceae (Vogel 1954, Collins and Rebelo 1985). When the full range of adaptive radiation of pollination systems are compared within each of these families it becomes obvious that shifts towards hairy scarabs form a portion of the unusually labile pollination systems indicative to southern Africa.

Families of petaloid monocots, eudicotyledonous herbs (Table 2) and generalist species (Table 3) indicate that the exploitation of beetles as pollen vectors has been a continuous process from the mid-Cretaceous through the present era. This is not to suggest, of course, that generalist systems in Australia and North American *Calochortus* spp. are

"evolving towards" more specialized modes of beetle-pollination. What is important is that generalist species exploit beetle families (e.g. Buprestidae, Cantharidae, Carabidae, Cleridae etc.) absent or uncommon in most magnoliids and basal monocotyledons. This suggests novel pollinator-flower interactions that could have evolved relatively late in the Tertiary.

Therefore, future lines of research must consider both the paleontology and molecular taxonomy of beetle-pollination. Unfortunately the recent treatment of fossils of anthophilous insects, to determine the radiation of insect-flower interactions (Grimaldi 1999), is inadequate. Grimaldi ignored fossils of Coleoptera claiming that beetle-pollination is of little significance to the story of pollination as it evolved as part of a generalist system now in decline. Discrediting the role of beetles as early pollen vectors in order to inflate the role of Diptera is counterproductive. In contrast, this review makes it abundantly clear that cantharophily is often a specialized system in extant plants and beetles remain keystone pollinators in certain ecosystems. While it is difficult to examine mouth-parts of beetle fossils and impossible to determine an extinct insect's ability to recognize floral colors and scents that is no reason to ignore the age and origin of a potentially anthophilous lineage within such families as the Curculionidae, Scarabaeidae, Staphylinidae etc.

In contrast, Farrell (1998) found a positive correlation between the phylogenetic history of diversity in phytophagous beetles and the phylogenetic history and diversity in vascular plants. Mapping pollination systems over a plant lineage can be extremely valuable as it indicates both the history and direction of shifts among allied plant species (Goldblatt et al. 1995, Johnson et al. 1998). While there are huge gaps in our knowledge of beetle-pollination, compared to predatory beetle-host plant interactions, there may be value in phylogenetic treatments of beetle-pollinated plants *vs.* their pollinators. For example, does the pollination of some modern palms and members of the Annonaceae

by curculionids (Table 2) have an older history than the pollination of some Myrtaceae by buprestids?

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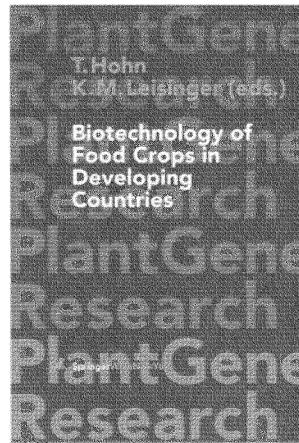
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